A STUDY ON DIAGNOSIS OF HELICOBACTER PYLORI INFECTIONBY CULTURE AND MOLECULAR METHODS FROM GASTRIC BIOPSY SPECIMENS AND SEROLOGICAL ASSAYS IN PATIENTS WITH PEPTIC ULCER DISEASE

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BONAFIDE CERTIFICATE

This is to certify that this dissertation work entitled **"A STUDYON DIAGNOSIS OF HELICOBACTER PYLORI INFECTION BY CULTURE AND MOLECULAR METHODS FROM GASTRIC BIOPSY SPECIMENS AND SEROLOGICAL ASSAYS IN PATIENTS WITH PEPTIC ULCER DISEASE"** is the original bonafide work done by **DR. VIJI S.,** Post Graduate Student from 2016 to 2018 under guidance and supervision in the Institute of Microbiology, Madras Medical College, Chennai- 600003, in partial fulfillment of the requirement of M.D MICROBIOLOGY degree Examination of The Dr.M.G.R. Medical University to be held in MAY 2019.

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I **DR. S.VIJI,** Post Graduate , Institute of Microbiology, Madras Medical College, solemnly declare that the dissertation titled **"A STUDY ON DIAGNOSIS OF HELICOBACTER PYLORI INFECTION BY CULTURE AND MOLECULAR METHODS FROM GASTRIC BIOPSY SPECIMENS AND SEROLOGICAL ASSAYS IN PATIENTS WITH PEPTIC ULCER DISEASE"**Submitted by me for the degree of M.D is the record work carried out by me during the period of OCTOBER 2017- SEPTEMBER 2018 under the expert guidance and supervision of **Prof. Dr. U. UMADEVI M.D.,** Professor, Institute of Microbiology, Madras Medical College, Chennai. The dissertation is submitted to The Tamil Nadu Dr. M.G.R Medical University, Chennai towards partial fulfillment of requirement for the award of M.D., Degree (Branch IV) in Microbiology examination to be held in May 2019.

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https://en.wikipedia.org/wiki/H_pylori
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CERTIFICATE II

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Introduction

INTRODUCTION

Helicobacter pylori was discovered by Drs.Marshall and Robin Warren[23] of Perth in 1982. Warren andMarshall were awarded the 2005 Nobel Prize in Physiology or Medicine.*Helicobacter pylori* is a Gram negative, microaerophilic bacterium found usually in the stomach. It is a curved motile rod found in the deeper portion of the mucous gel coating the gastric mucosa. It is extraordinary among bacteria in it's ability to colonize and persist among this niche for decades despite host defences and gastric acidity.

However, over 80% of the individuals infected with the H.pylori are asymptomatic, and it may play an important role in the natural stomach ecology. *H. pylori* infection is more prevalent in developing countries. The nitrate conversion feature of the bacteria acts as a factor in the causation of infection ranging from mild gastritis to peptic ulcers[24] and even gastric malignancies, such that the International Agency for Research on cancer has declared this pathogen as an independent carcinogen. In addition to the gastric disorders, *H.pylori* is also associated with of this infection with cardiovascular diseases[25](due to virulence factor of Cag gene) and metabolic syndrome [26](due to the release of Interleukins) are being investigated.

H. pylori infection is detected by several diagnostic modalities. These are classified into 1. Endoscopic or invasive tests and 2.Non Endoscopic or non – invasive tests. The Endoscopic tests are Rapid urease test, Histopathological examination, Culture (Gold standard) and Polymerase chain reaction(PCR). The

non– invasive tests tests are antibody detection (IgG),Stool antigen test and carbon labelled (^{13}C or ^{14}C) Urea breath test. The selection of tests are based on the clinical condition and laboratory resources to diagnose the infection.

H. pylori infection is detected immediately from gastric biopsy tissues by Rapid urease test (RUT) and Gram stain or Giemsa stain. The gastric biopsy specimens are also used for histopathological examination (HPE) .

Though Culture is the gold standard , it is probably difficult to isolate the *H.pylori* in majority of the cases. But the advantages are its high specificity and additionally determination of antibiotic susceptibility test from the culture isolates.

However , this organism being fastidious and microaerophilic in nature , culture methods has limited role in primary diagnosis.

Detection of IgG antibodies in serum is used for diagnosis of *H.pylori* colonization.

It induces both local and systemic immune response. Serum Anti-Helicobacter pylori IgG

Antibody (HpIgG) titre was measured by using quantitative ELISA. Serology is sensitive for primary diagnosis but not useful for assessing post treatment status of the infection[27].

The Urea breath test (UBT) relies on the urease activity of *H.pylori* which converts urea into carbon dioxide ,which is detected in exhaled breath after 10 min[16].The test has excellent sensitivity, because it represents the major portion of stomach. Unlike serology , it is useful in determining the success of eradication therapy. Hence it is useful screening test, additional advantage is that it is non invasive test. Limitations of tests are that it is non specific test and its high cost.

Molecular methods like PCR (Polymerase chain reaction) are also very useful in identification of H. pylori in gastric biopsy samples. PCR also used to detect CagA and VacA virulence genes in gastric biopsy samples[28]. The potential advantage of PCR includes high specificity, quick results and ability to identify different strains of bacteria for pathogenic and epidemiologic studies. The major disadvantage of the molecular methods are they cannot be performed in resource limited settings.

In the present study, the following parameters , namely rapid urease test(RUT), Gram stain, Giemsa stain, culture method, and serology (HpIgG) were used to detect the presence of *H.pylori* in gastric biopsy samples which were subjected to HPE for pathological classification of gastric lesions. The diagnosis of *H.pylori* infection was supplemented with PCR in randomly selected 50 gastric biopsy samples.

The analysis was done with the results of *H.pylori* infection by various methods in correlation with the results of histopathological examination.

Aims &objectives

AIMS & OBJECTIVES

1. To identify the *Helicobacter pylori* in gastric biopsy samples from patients with clinical diagnosis of gastroduodenal disease.

2. To compare the various tests like microscopic examination of Gram stain, Giemsa stain smears, Rapid urease test and Histopathology correlation with culture and molecular methods for identification of *H.pylori.*

3. To evaluate antibody IgG response to *H.pylori* by ELISA.

4. To perform the molecular detection of *H.pylori* from the gastric biopsy samples.

Review of Literature

REVIEW OF LITERATURE

HISTORICAL PERSPECTIVES[29]

The presence of gastric spiral bacteria was first reported in 1893. Spiral bacteria were demonstrated for the first time, in the human stomach in 1906. In 1924 the presence of urease activity in the human stomach was documented. The bacterial source of gastric urease was confirmed in 1968. Bacteria were reported in association with gastritis in 80% of gastric resection specimens from patients with gastric ulceration.

The modern era was heralded in 1981, when Barry Marshall, began a clinical research project with Robin Warren, in the Royal Perth hospital,western Australia. Subsequent attempts to culture the bacilli weresuccessful until April 1982, when during the easter weekend, the plates were unintentionally incubated for 3 to 7 days and colonies were visible.

The association between *H.pylori* with gastritis was first presented at the Royal Australian college of Physicians on 22 october 1982 and published in letter form in 1983.

These bacteria was previously called as" Campylobacter pyloridis", then changed to Campylobacter pylori in 1987 due to grammatical reasons. Then it was shown that C. pylori did not belong to the genus Campylobacter and a new genus name was suggested in 1989[30].

The association with peptic ulceration and possibly with gastric adenocarcinoma, was initially suggested by Marshall et al[31].

The Epidemiological and interventional studies were conducted due to availability of reliable diagnostic studies, such as Rapid urease test (RUT), Urea breath test and serology.

The European study group was formed in Copenhagen in 1987 to study the role of the bacteria in gastroduodenal disease. The first long - term clinical trial of treatment aimed at eradicating *H.pylori* in patients with duodenal ulceration was reported in1987.

The relation between the *H.pylori* and gastric Adenocarcinoma and MALT (Gastric mucosa associated lymphoid tissue) Lymphoma was reported in 1991.

Subsequent studies assessed the role of the organism in Gastro oesophageal reflex disease, patients receiving long ─ term acid suppressing medication, Paediatric populations and non - ulcer dyspepsia.

In 1994, *H.pylori* was recognized as a Grade 1 (definite) carcinogen and the National Institute of Health Consensus Developement Conference statement recommended that all patients who are found to have gastric or duodenal ulceration and concurrent *H.pylori* infection should receive treatment aimed at eradicating the bacterium[81].

In 1997, it was strongly recommended by a European panel that patients with *H.pylori* infection and peptic ulcer ,low grade mucosa associated lymphoid tissue lymphoma. Severe Macroscopic or Microscopic gastritis or recently resected early gastric cancer should receive a Proton pump inhibitor based triple therapy to eradicate the infection.

DEFINITION OF GENUS

Helicobacter pylori are helical, S shaped or curved Gram negative rods, 0.5─ 1.0µm wide by 2.5 ─ 5.0µm length. Motility is rapid and shows darting by means of single or multiple unipolar, bipolar, or lateral sheathed flagellar filaments of *Helicobacter* composed of two copolymerized flagellins,FlaAand FlaB. They are microaerophilic and non-sporing with respiratory metabolism. They are Catalase positive (except H.canis)[33] , Oxidase positive , non saccharolytic and can convert to viable but non culturable coccoid bodies.
They also have the ability to produce biofilm formation.

HABITAT OF THE BACTERIUM[8]

The surface of the human stomach mucosa is major habitat of *H.pylori*. It's natural niche appears to be themucosa lined surface of the non- acid secreting area close to the pyloric sphincter (ie) the antrum[8].

Almost all isolations are from gastric biopsy specimens, but the organism has occasionally been detected in gastric juices, dental plaque [34], bile and faeces.

MORPHOLOGY OF THE BACTERIUM[35]

Helicobacter pylori cells are take the form of curved or S shaped Gram negative rods,0.5 – 0.9 μm width and 3um length with wavelength of about 2.6μm. In agar cultures spiral forms are less obvious and cells appear more as singly curved rods. *H.pylori* undergo coccal transformation on exposure to air within 1 to 2 hours at room temperature, and in this state it fails to grow on subculture[35]. Such coccoid forms don't appear to be virulent, (Eaton et al,1995).

H.pylori appears as spiral , bluntly rounded ends with 4 to 8 sheathed, unipolar flagella under Electron Microscope. The sheath is continuous with the outer membrane of the cell wall. Some flagella have a terminal bulb. A glycocalyx - like material surrounding the cell is also apparent.

CELL WALL COMPONENT AND ANTIGENIC STRUCTURE[39]

Helicobacter pylori have the typical cell wall structure of Gram negative bacteria. The fatty acid profile of H.pylori is distinctive and is characterised by long chain fatty acids composed predominantly of tetrad - canoic (14:0) and 19-carbon cyclopropane (19:0) acids (Moran 1995)[39].

H.pylori contains atleast 5 outer membrane proteins (OMP) ranging from 48 to 67 kDa that have pore forming ability. The various strains share the same lipopolysaccharide (LPS) core antigens but differ in their side chain antigens .

Pathophysiology

Adaptation to the stomach'sacidic environment

To avoid the acidic environment of the interior of the stomach lumen, *H.pylori* uses its flagella to burrow into the mucus lining of the stomach to reach the epithelial cells underneath, where it is less acidic. *H.pylori* is able to sense the pH gradient in the mucus and move towards the less acidic region (chemotaxis). This also keeps the bacteria from being swept away into the lumen with the bacteria's mucus environment, which is constantly moving from its site of creation at the epithelium to its dissolution at the lumen interface.

In addition to using chemostaxis to avoid areas of low pH, H.pylori also neutralises the acid in its environtment by producing large amounts of urease, which breaks down the urea present in the stomach to carbon dioxide and ammonia.

H.pylori is found in the mucus, on the inner surface of the epithelium, and occasionally inside the epithelial cells themselves. It adheres to the epithelial cells by producing adhesions, which bind to lipids and carbohydrates in the in the epithelial cell membrane.

Inflammation, gastritis, and ulcer

H.pylori harms the stomach and duodenal linings by several mechanisms. The ammonia produced to regulate pH is toxic to epithelial cells, as are biochemicals produced by H.pylori such as proteases, vacuolating associated cytotoxin A (VacA). This damages epithelial cells, disrupts tight junctions and

causes apoptosis, and certain phospholipases. Cytotoxin associated gene CagA can also cause inflammation and is potentially a carcinogen.

Colonization in the stomach by *H.pylori* can result in chronic gastritis. *Helicobacter* cysteine – rich proteins (Hcp), particularly HcpA (hp0211), are known to trigger an immune response, causing inflammation.

Ulcers in the stomach and duodenum result when the consequences of inflammation allow stomach acid and the digestive enzyme pepsin to overwhelm the mechanisms that protect the stomach and duodenal mucous membranes. The location of colonization of *H.pylori* , which affects the location of the ulcer, depends on the acidity of the stomach.

The inflammatory response caused by bacteria colonizing near the pyloric antrum induces G cells in the antrum to secrete the hormone gastrin, which travels through the bloodstream to parietal cells in the fundus. Gastrin stimulates the parietal cells to secrete more acid into the stomach lumen, and over time increases the number of parietal cells, as well. The increased acid load damages the duodenum, which may eventually result in ulcers forming in the duodenum.

When *H.pylori* colonizes other areas of the stomach, the inflammatory response can result in atrophy of the stomach lining and eventually ulcers in the stomach. This also may increase the risk of stomach cancer.

Following attachment of H.pylori to stomach epithelial cells, the type IV secretion system expressed by the cag PAI injects the inflammation – inducing

agent peptidoglycan, from their own cell walls into the epithelial cells. The type IV secretion apparatus acts on cagA into the stomach's epithelial cells where it disrupts the cytoskeleton, adherence to adjacent cells, intracellular signalling, cell polarity and other cellular activities. This may lead to increased risk of gastric cancer.

NSAID - INDUCED DISEASE[49]

NSAIDS like Aspirin and analgesics may cause mucosal injury (ulcers and erosions).Prostaglandins play a critical role in maintaining gastroduodenal mucosal integrity and repair.It therefore follows that interruption of prostaglandin synthesis can impair mucosal defense and repair, thus facilitating mucosal injury via systemic mechanism. Animal studies have demonstrated that neutrophil adherence to the gastric microcirculation plays an essential in the initiation of NSAID – induced mucosal injury.

Topical NSAIDs can also alter the surface mucous layer, permitting back diffusion of H+ and pepsin , leading to further epithelial cell damage. Moreover, enteric – coated or buffered preparationsare also associated with peptic ulceration.

Survival of *H.pylori*

The pathogenesis of *H.pylori* depends on its ability to survive in the harsh gastric environment characterised by acidity, and attack by phagocytosis accompanied by release of reactive oxygen species. This oxidative stress response induces potentially lethal and mutagenic oxidative DNA adducts in the *H.pylori* genome.

H.pylori able to surviving the DNA damage induced by oxidative stress appears supported by transformation mediated recombinational repair which contribute to successful infection.

GENES INVOLVED IN VIRULENCE AND PATHOGENESIS[6]

Study of the *H.pylori* genome is centered on attempts to understand pathogenesis,the ability of this organism to cause disease. The CagA gene codes for one of the major *H.pylori* virulence proteins. CagA pathogenecity island (PAI) which code for type IV secretion system is associated with increased incidence of peptic ulcer disease and Adenocarcinoma[9].

The VacA gene is seen in all *H.pylori* strains has been associated with increased gastric damage[37].

The low GC - content of the cag PAI related to the *Helicobacter* genome suggests the island was acquired by horizontal transfer from another bacterial species.

VacAs1 and VacAs2 code for toxigenic and non toxigenic types respectively. Other important genes are babA 1, babA2 and babB[11] which code for blood group antigen binding adhesion molecule, flagellin genes flaA and flab, iceA 1 gene code for restriction endonuclease,OipA that codes for a protein which induces IL─8 and genes ureA, ureB, E,F,G that code for urease[28].

H.pylori consists of large diversity of strains,and the genome of three have beencompletely sequenced. The genome of the strain "26695" consists of about 1.7 million base pairs, with some 1,576 genes.

Susceptibility to antimicrobial agents

H.pylori is sensitive to penicillins, (including benzyl penicillin), cephalosporins,tetracycline, erythromycin, rifampicin, aminoglycosides and nitrofurans, but resistant to nalidixic acid, though sensitive to the more active quinolones such as ciprofloxacin. *H.pylori* is usually Susceptible to metronidazole, and are sensitive to colloidal bismuth compounds commonly prescribed for gastric disease in concentrations easily attainable in the stomach[39]. The proton pump inhibitor omeprazole has mild in vitro activity against *H.pylori*. 1% bile salts are also inhibitory.

Epidemiology of Helicobacter

H.pylori infections were usually acquired in early childhood in all countries. However, the infection rate of children in developing nation is higher than developed countries. people infected at an early age are likely to develop more intense inflammation that may be followed by atrophic gastritis with high subsequent risk of gastric ulcer, gastric cancer, or both. Acquisition at an older age brings different gastric changes more likely to lead to duodenal ulcer.

Infectious dose[40]

Data concerning infectious dosecome from the first successful volunteer infection, in which a dose of 10^9 organisms in a small liquid feed was used.

H.pylori infection is difficult to diagnose, so Animal models, like primates , and related studies with other Helicobacter [26] might help to diagnose the natural infection.

Modes of transmission

By direct contact;

The major modes of transmission is uncertain, may through oral-oral, gastro- oral, and faecal- oral routes[41].

By water;

Though examples among children in peru, South America suggest a role for water as a vehicle, it does not project it as main route of acquisition[42].

By fomites;

H.pylori infection was transmitted by endoscope[43] as iatrogenic, acute mucosal lesion syndrome in japan and out breaks of achlorhydria in U.S restriction enzyme analysis of bacterial DNA demonstrated as identical strains in infected individuals were examples.

By animal reservoir or foods

H.pylori may found in cats , as reservoir for human infection , but not definitely.

By other modes

There was a possibility of re-infection by person to person transmission between spouses. It is also transmitted through mouth as a reservoir for re-infection, even though samples are often culture negative.

Gastro –oral transmission is common among children. Several reports relate to spread from a faecal source. The organism can be detected in faeces by PCR.

Variation in the age of acquisition of *H. pylori* infection

H.pylori infection shows marked geographical variation . It is more common among children under 15 years of age than adults. *H.pylori* infection has been suggested to protect against obesity and childhood asthma.

Risk factors for *H.pylori* infection[44]

Low socio economic status and environmental factors like preserved food with salt promote pangastritis[44].

Blood group O is postulated to contribute to *H.pylori* adherence and to H.pylori related duodenal ulceration. Blood group A, on the other hand, has been associated with the diffuse type of gastric cancer[10].

ABO blood group, cigarette smoking, alcohol and diet are non-infectious risk factor for gastric cancer. Clustering of infection within families has been commonly, but not uniformly observed[53].

The seropositivity is high among children in developing countries. Seroprevalence of *H.pylori* infection is similar in males and females. There is increased risk of infection in dentists, gastroenterologists and endoscopists .

H.pylori gastritis – related hypochlorhydria and iron deficiency anaemia both of which can have major deleterious effects on physical and intellectual growth of children especially in developing countries.

Risk of re-infection

The minimum period is 4 weeks after treatment is the cut off time to find out the re-infection. This treatment failure isdue to persistence of same strain[54].

Pathogenesis of clinical syndromes[46]

H.pylori gastritis is associated with several important pathologic conditions including;-

1.Duodenal ulcer disease

2.Gastric ulcer disease

3.Gastric adenocarcinoma from antrum and cardia of stomach

4. Gastric lymphoma

1. Duodenal ulcer disease

About 90% of duodenal ulcer disease is associated with *H.pylori* infection. It occurs more often in the first part of the duodenum, with, 90% located within 3cm of the pylorus.

Ulcers are sharply demarcated, with depth at times reaching the muscularis propria. The presence of Cag (cytotoxin associated gene) pathogenicity island ,and production of an active vacuolating cytotoxin. Vac A are most commonly associated with H.pylori strains. The island encodes a type IV secretory system of Cag encoded protein induces the epithelial cell to undergo several changes including the secretion of pro- inflammatory cytokines, which leads to increased gastric inflammation. Cag A is also highly immunogenic and anti- Cag A antibody detection can be used as a serum test for the presence of the island.

The vacuolating cytotoxin ,VacA , is a pore - forming toxin that increases epithelial permeability and causes massive epithelial cell vacuolation in vitro.

However, certain VacA, genotype is associated with increased prevalence of peptic ulcer disease.

There were an recent research in other virulence factors include an adhesion, BabA, a bacterial outer membrane pro inflammatory protein, OipA, and a restriction enzyme, Ice A, and its associated methylase. Other bacterial factors are also important in pathogenesis of colonization and the induction of infection, such as enzyme urease and the ability to adhere to gastric mucosa.

2.Gastric ulcer disease

H.pylori associated gastric ulcers usually common in mucosal junction between antral and corpus type tissues, mostly in lesser curvature. It is mostly occurs in pan-gastritis patients rather than antral gastritis and are not associated with increased acid output. Their pahogenesis is uncertain, but infection with virulent strains and smoking increased risk.

Gastric ulcers have been classified based on their location; Type I occur in the gastric body and tend to be associated with low gastric acid production. Type II occur in the antrum and the gastric acid can vary from low to normal. Type III occur within 3cm of the pylorus and are commonly accompanied by duodenal ulcers and normal or high gastric acid production; and type IV are found in the cardia and are associated with low gastric acid production.

3.Gastric adenocarcinoma

Gastric adenocarcinoma is usually arises in patients with pangastritis. Both Cag and cytotoxic strains are most likely associated with carcinoma than other strains. Host genetics are also important. People with genetic polymorphisms that lead to high level secretion of pro – inflammatory cytokines IL- 1 in response to bacterial infection are more likely to develope gastric cancer. Intestinal and diffuse type of gastric carcinoma occurs due to *H.pylori* infection. Intestinal type of gastric carcinoma is thought to be occur by a step-wise process from superficial gastritis through atrophy to intestinal metaplasia, dysplasia and ultimately carcinoma.

4. Gastric lymphoma

Gastric lymphoma primarily arise in lymphoid tissue present in the *H.pylori* infected stomach. Low grade lymphomas regressed by *H.pylori* eradication treatment.

In duodenal ulcer patients gastric metaplasia is due to binding of *H.pylori* to the epithelial surface and produce local injury secondary to the host response. *H.pylori* antral infection could lead to increased acid production, increased duodenal acid, and mucosal injury. It also induces secretion of proinflammatory cytokines (IL-8,TNF, and IL-1) on parietal cell. The over all effect of *H.pylori* on gastrointestinal tract is variable and determined by host and microbial factors.

The gastric and duodenal pathology is observed by the type and distribution of gastritis. The presence of antral gastritis is associated with duodenal ulcer formation. The corpus gastritis predisposes to the developement of gastric ulcer, gastric atrophy, finally gastric carcinoma.

CLINICAL FEATURES

1. Acute *H.pylori* infection

2. Chronic *H.pylori* infection

1. **Acute *H.pylori* infection**[55]

The most common causes of acute gastritis are infectious. Acute infection with *H.pylori* induces gastritis and are most commonly acquired in childhood, whether the colonization is symptamatic or asymptamatic is not known. It is presented with sudden onset of epigastric pain and nausea followed by vomiting and finally resolution of symptoms by the end of the week.

Acute gastritis also associated with other organisms like *Staphylococci, Streptococci, Escherichia coli, Proteus, and Haemophilus species*. Other types of infectious gastritis may occur in immunocompromised individuals such as AIDS patients.

1. **Chronic *H.pylori* infection**

Chronic gastritis is identified by histologically as severe glandular destruction,with atrophy and metaplasia. The early phase of chronic gastritis is superficial gastritis. The inflammatory changes are limited to the lamina propria of the surface mucosa. The next stage is atrophic gastritis. The inflammatory infiltrate extends deeper into the mucosa, with destruction of glands. Gastric glands may undergo morphologic transformation in chronic gastritis into intestinal metaplasia. Intestinal metaplasia is an important predisposing factor for gastric cancer.

Chronic *H.pylori* infection is characterized by chronic active gastritis, but usually asymptamatic. It is only symptomatic, when complications like duodenal ulcer, gastric ulcer or gastric cancer develops.

LABORATORY DIAGNOSIS

The two major categories for diagnosis of *H.pylori* are endoscopic or invasive tests and non endoscopic or non invasive tests.

Specimen collection

Gastric biopsy tissue, blood, and stool etc...collected for diagnosis of *H.pylori* infection. Ideally the patient should not consume proton pump inhibitors for 2 weeks prior to endoscopy.

TYPES	NAME OF THE TEST	METHOD OF ORGANISM IDENTIFICATION
INVASIVE TESTS	Rapid urease test	By urease production
	Histology	By morphology and location
	Culture	By biochemical reactions
	Polymerase chain reaction	By genetic sequencing
NON INVASIVE TESTS	Antibody detection	By immunologic response
	Urea breath test	By urease production
	Stool antigen assay	By coproantigen

Endoscopic or invasive tests

The stomach is assessed by fiber optic endoscopy, and biopsy specimens are obtained. Using two contrast strains, topical acriflavine and intravenous fluorescein, with a confocal endomicroscope , endoscopists were able to detect the clusters of *H.pylori* on the surface and in the deeper layer of the gastric epithelium[53]. This technique enabled detection of by surface microscopy for the first time.

It is possible that gastric juice obtained by a nasogastric tube allows the detection of *H.pylori* by culture, staining, urease test, and PCR, but it is less reliable than gastric biopsy specimens. The string test can also be used to obtain gastric mucus, However , the most attractive seems to be an extendable oro-gastric brush contained in a plastic tube. The brush is swallowed, extended into the stomach to brush the mucosa three or four times, retracted in the protected sleeve, and withdrawn from the patient. This method is rapid and considered as gold standard test for the diagnosis of *H.pylori* infection.

Transport of biopsy specimens[56]

H.pylori is an microaerophilic fragile organism, and must be protected from dessication and contact with oxygen and room temperature. It is mandatory to place them in a saline solution for short –term transport (4 hrs maximum) or in a transport medium, usually consisting of semisolid agar ,maintained at 4°C or at deep freezer. A commercially available medium porta – germ *pylori* is effective for this purpose. Storage at 4°C in a medium containing 20% glycerol also led to *H.pylori* recovery from gastric biopsy specimens.

Grinding of the biopsy specimens

Comparision of culture performed with and without grinding showed a higher numberof colonies after grinding, for this reason grinding of the biopsy specimen is mandatory[57].

Culture media

There are selective and non selective media available for detection of *H.pylori*. the media components include an agar base, growth supplements and selective supplements. Most agar bases are satisfactory for growing *H.pylori*. e.g., brain heart infusion agar, Columbia agar. Concerning the growth supplement, it is mandatory to add blood or serum, which includes numerous nutrients (vitamins and oligo elements, etc..) which enhance *H.pylori* growth.

The proportion of blood or serum can be 5%,7% or preferably, 10%. Red blood cells can be lysed for these growth substances o be more readily available, animal blood, eg., sheep or horse blood, can be added. Other growth supplements such as starch[58], bovine serum albumin and cyclodextrins, which are cyclic oligosaccharides produced from starch by enzymatic treatment retaining the same properties as starch, are employed.

Celini et al, proposed a blood – free medium supplemented with isovitalex (2%) and hemin (10mg/litre). They also added urea (20 g/litre) and a pH indicator(phenol red) to identify the urease – positive colonies. *H.pylori* grows

best at a slightly acidic pH (5 to 6), in agreement with its ecological niche, the mucus layer, where a pH gradient exists[62].

Different selective supplements containing antimicrobial compounds have been proposed ;vancomycin or teicoplanin to inhibit gram – positive bacteria; polymyxin , nalidixic acid, colistin, trimethoprim, or cefsulodin to inhibit gram negative rods; and nystatin or amphotericin B to inhibit fungi.

Another supplement which may be helpful to readily identify *H.pylori* colonies is 2,3,5 – triphenyltetrazolium chloride (40mg/ litre). This compound is reduced by *H.pylori* to insoluble red formazan complexes, resulting in easily distinguished pigmented golden colonies.

Non selective media such as chocolate agar, Brain heart infusion agar with 5% horse blood, Brucella agar with 5% sheep blood and tryptone soya agar with 5% sheep blood can be used. Selective media include Skirrows campylobacter medium and Brain heart infusion agar with vancomycin(6ug /ml), nalidixic acid (20ug/ ml), and amphotericin (2ug/ml) have given good recovery.

Helicobacters are microaerophilic and capnophilic. Several systems can be used to achieve a microaerobic atmosphere systems , such as microaerobic cabinet or an incubator with an adjustable gas level, to jars in which the adequate atmosphere is created with an automatic apparatus or with H_2- CO_2- generating packs. The atmosphere in jars will vary according to the quantity of bacteria consuming oxygen; therefore, the gas pack should bechanged every other day,

while *H.pylori* growth is possible in a candle jar, it takes a longer time and results in small colonies.

The optimum temperature is 37°C, testifying to the adaptation of this bacterium to humans. For primary culture under optimal conditions. Colonies may appear after 3 days and are at their optimum on day 4. However, in the case of negative culture, a 7 to 10 days incubation is recommended to ensure that the result is negative; If only a few organisms are present, this time lapse may be necessary to visualise the colonies.

In contrast, subcultures only take 2 to 3 days. When few colonies are present, the recommendation is to subculture by plating the colonies on a small area of the agar plate. It is important to remember that once *H.pylori* reaches its growth plateau, it becomes coccoidal and loses its viability, most likely due to a lack of adequate nutrients.

Broth culture[39]

Brain heart infusion or Brucella broth with 1-10% fetal calf serum may be preferable for studies on physiology and metabolism.

Identification of Helicobacter pylori in culture[49]

The growth of small, circular, smooth grey and translucent colonies observed after 3 to 4 days on the selective media plated with gastric biopsy specimens is an important criterion for *H.pylori* identification.

Gram staining of the colonies reveals gram negative curved rods, the spiral forms being less obvious. The characteristic gull wing is seen in broth cultures. Motility is demonstrated in broth cultures and is weak when grown on agar.

The identification of culture consists essentially of certain enzymes; cytochrome oxidase, catalase, and urease which are positive[49].

The commercial RUT dry test kit manufactured by Gastro cure systems, Kolkatta ,India was used to identify urease production from gastric biopsy tissues. The colour change from yellow to red indicates positive[36].

Histopathological diagnosis[47]

H.pylori can be identified with haemotoxylin and eosin ,but the bacteria can be more reliably seen with special stains such as acridine orange, modified Giemsa, cresyl violet or Warthin – starry stains.

The typical morphology of *H.pylori* is a comma shaped bacillus observed on the epithelial surface.

Gram staining of the touch smear of the biopsy specimen by rubbing it forcefully on a glass slide was used to confirm the presence of gram negative spiral rods indicates presence of *H.pylori*.

Warthin - starry silver stain demonstrates *H.pylori* clearly as spiraled black rods against a yellow background. In Giemsa stained sections, the organisms are clearly visible as Giemsa – positive (dark blue) spiraled rods.

Urease tests

The discovery that *H.pylori* were a strong urease producer was made by Langenberg et al and was used for diagnosis by McNulty and Wise. When a biopsy specimen containing *H.pylori* is introduced into a urea rich medium, the urease hydrolyses the urea down into carbon dioxide and ammonia. The ammonium ion increases the pH , and a pH indicator, e.g., phenol red, changes color, in this case from yellow to red.

The commercial RUT dry test kit manufactured by Gastro cure systems, Kolkatta, India, used to identify urease production from gastric biopsy tissues.

Commercial Kits[60]

The first generation commercial kits were agar based, e.g., the CLO test. The new generation kits introduced in 1995 are strip based tests.

In the first study, Rogge et al [60], compared this new test to the CLO test which showed 99% sensitivity and 95% specificity after 2 hours, which is superior to those of the CLO test.

One step antibodies to H.pylori test by SDBIOLINE *H.pylori* Kit

The SDBIOLINE *H.pylori* test contains a membrane strip, which is precoated with *H.pylori* capture antigen on test band region. The *H.pylori* antigen colloid gold conjugate and serum sample moves along the membrane chromatographically to the test region (T) and forms a visible line as the antigen – antibody gold particle complex forms with high degree of sensitivity and specificity.

The SDBIOLINE *H.pylori* test is a rapid test for the qualitative detection of antibodies of all Isotypes (IgG, IgM, IgA, etc) specific to *Helicobacter pylori* in human serum, plasma orwhole blood.

Procedure

1. Remove the test device from the foil, and place it on a flat, dry surface.
2. Transfer 10µl of serum or plasma. Add 3 drops of assay diluents (approximately 110µl) and start the timer.
3. Interpret test results at 10 minutes. The result should not be interpreted after 10 minutes.

Interpretation of the test

- **Negative Result ;** The presence of only one purple colour band within the result window indicates a negative result.

- **Positive Result ;** The presence of only two colour bands ("T" band and "C" band) within the result window, no matter which band appears first, indicates a positive result.
- **Invalid Result :** If the purple colour band is not visible within the result window after performing the test, the result is considered invalid.

Polymerase chain reaction (PCR)[17]

The PCR was developed in the 1980 and thereafter quickly applied to the detection of *H.pylori*. Its application in the field of *H.pylori* concerns not only the detection of the bacterium but also its quantification and detection of specific genes relavant to pathogenesis (CagA)[18] and specific mutations associated with antimicrobial resistance.

The first target used were the genes of the ureaseoperon; ureA and glmM, or the 16S rRNA gene.

Two main pathogenic factors the Cag PAI and the plymorphism of the VacA gene and other genes involved in adherence (babA2 ,sabA) or in pathogenicity (oipA, dupA, ,iceA)[11]can also be detected by PCR. The new real time PCR technique[17] is considered a breakthrough as it allows quantification and detection of point mutation associated with antibiotic resistance.

Non – invasive test

The first method used was serology. However, due to the difficulty in obtaining an optimal specificity, other methods have been proposed namely Urea breath test, stool antigen test, and most recently , detection of specific antibodies in urine or saliva.

Urea Breath Test (UBT)[16]

Upto 2 weeks before the test, you need to stop taking antibiotics, bismuth medicines such as pepto-bismol, and proton pump inhibitors (PPIs).

During the test, you swallow a special substance that has urea. Urea is a waste product the body produces as it breaks down protein. The urea used in the test has been harmlessly radioactive.

A solution of labelled urea ingested by the patient is rapidly hydrolysed by *H.pylori* urease, the labelled CO_2 is absorbed by the blood and exhaled in expired air. If the patient is not infected, most of the isotope is eliminated in urine without modification.

When (13C) urea is used , a specimen collection is performed before and 30 min after the ingestion. This method screens the major part of the stomach.

The 13C /12 C ratio is measured in both specimens, and the result is expressed as the difference between the two measurements. The need for a baseline value is due to the various amounts of 13C present in breath according to

patient diet. When (14C) urea is used, specimen collection occurs only 20 min after ingestion.

The test can identify almost all people who have *H.pylori.* It can also be used to check that the infection has been fully treated.

StoolAntigen Tests [14]

H.pylori culture from stools is not used as a routine diagnostic method. The first report of successful detection of *H.pylori* antigens in stools was made in 1997 by Kozak et al [14] who reported an enzyme linked immunosorbant assay (ELISA) performed on stools. This test was named *H.pylori* stool antigen test (HpSA).

H.pylori infection is usually acquired in early childhood. The stool test can detect traces of *H.pylori* in the feces. Because many studies support the hyphothesis of a fecal – oral route of infection.

***H.pylori* stool antigen test (HpSA)**

A fresh stool sample with the size of peanut was collected and stored at -20°C for analysis. The test is based on a sandwich EIA with antigen detection. This is qualitative test with a polyclonal rabbit anti-*H.pylori* antibody adsorbed to microwells as capture antibody.

First, 100µl of a diluted stool sample (10µl stool in a 0.5ml sample diluent) and thereafter, peroxidise – conjugated polyclonal antibody solution were added to the wells and incubated for 1 hour at room temperature. Unbound material was

removed by washing. After addition of a substrate solution, *H.pylori* antigen could be detected by a color change.

A stop solution was added and the absorbance was read at 450nm by a spectrophotometer. The results were interpreted as positive, negative and equivocal. Equivocal results should be repeated.

Serodiagnosis[15]

The diagnostic utility of serological assays to ascertain the colonization status of H.pylori by measuring specific IgG levels in serum and it's association with clinical disease have been studied by various investigators. It has been reported that the height of antibody titre correlates with the severity of gastritis[3].

H.pylori infection is a chronic condition and immunoglobulin G (IgG)[5] (subclass 1 and 4) is the predominant immunoglobulin class, even in children IgG are present at the mucosal level and detected in virtually all blood samples. IgM are rarely observed, merely because acute H.pylori infections are seldom available for study.

CALBIOTECH ELISAIgG KIT available to detect H.pylori infection. The performance ofan ELISA is largely dependent on the nature of the antigens used. The first antigens to be used were Whole cell sonicates.

Developement of point- of - care tests

They are essentially based on the diffusion of antibodies from a drop of serum or whole blood obtained by finger puncture through a membrane and an immunoenzymatic reaction.

The first test proposed had a very promising performance (sensitivity, 92%; specificity, 88%). However, the point -of –care tests have not been recommended.

Immunoblot analysis and detection of Cag A antibodies[7]

The immunoblot is most likely used as a second step technique to identify false-positive cases detected by ELISA, in which case, the criteria proposed by Nillson et al must be used. A commercial immunoblot test is now available, which is an important advance towards standardization.

Detection of H.pylori antibodies in urine[59]

Specific H.pylori IgG antibodies are eliminated in urine but at very low concentrations.

Detection of H.pylori antibodies in saliva[34]

Detection of IgG antibodies is used by ELISA or by immunoblotting by various commercial kits.

Barium contrast imaging[8]

If endoscopy is not available, for symptomatic patients, barium contrast imaging can be used to detect peptic ulcer disease.

Treatment[49]

The current recommended treatment for H.pylori eradication includes two antibiotics and an antisecretory drug, essentially a PPI, to which a bismuth salt can be added. The Triple therapy(I and II) is given as double dose of PPI (omeprazole, esomeprazole, pantaprazole, rabeprazole or lansoprazole) plus clarithromycin (500 mg twice a day (b.i.d) or amoxicillin (1 g b.i.d) and metronidazole (500 mg twice a day (b.i.d) for first 7 days and then for 10 – 14 days.

The Quadruple regimen (III) contains omeprazole 20 mg twice a day (b.i.d) plusBismuth subsalicylate 2 tab Q.i.d., plus metronidazole 500 mgtwice a day (b.i.d) plus tetracycline 500 mg Q.i.d for 14 days.

Antimicrobial susceptibility testing of the isolates[35]

H.pylori can acquire resistance to antimicrobial agents used to treat the infection, and therefore susceptibility testing is important in the management of the infection.

H.pylori is intrinsic resistant to glycopeptides, cefsulodin,polymyxins, nalidixic acid, trimethoprim, sulphonamides,nystatin, amphotericin B, and cycloheximide. Some of these are used as selective agents in isolation media. H.pylori acquire resistance by mutation.

Susceptibility Testing Methods;

1. Phenotypic methods;

Agar dilution method;

It is usually considered the reference method to compare other techniques, has been proposed by the Clinical Standard Institute (CLSI) as the method to be used for H.pylori clarithromycin susceptibility testing[52].

Broth dilution method;

It has rarely been used for H.pylori because of the difficulty in growing, this bacterium is in broth[50].

Disk diffusion testing;[7]

The disk diffusion method is the simplest and most economic for susceptibilitytesting.

H.pylori antibiotic susceptibility test was done by using Kirby – Bauer disc diffusion method on Muller – Hinton agar plate supplemented with 10% sheep blood. A standard inoculums of H.pylori culture was suspended in BHI broth. The

turbidity was adjusted equal to McFarland 3. The inoculums was seeded onto Muller – Hinton blood agar plate using sterile cotton wool swab antibiotic discs with the following drug contents;

Metronidazole (5µg), Amoxicillin (10µg), Tetracycline (30µg), Erythromycin (15µg), Levofloxacin (5µg), Norfloxacin (5µg), Cotrmoxazole (10µg), were placed on the plates.

The plates were incubated at 37°C in CO2 jar for 3 – 4 days. The results were interpreted as per Clinical and Laboratory Standards Institue (CLSI) 2018 guidelines.

E test;

The latest method has the advantage of being a quantitative method with a direct expression of MICs, and furthermore, it is adapted to slow growing bacteria like H.pylori.

Genotypic detection of resistance;

H.pylori resistance is essentially due to chromosomal mutations which can be easily detected with molecular tests. Resistance to macrolides, fluroquinolones , tetracycline and metronidazole can be detected by RT-PCR. Fluroscent -in-situ-hybridization (FISH) also has been used to detect clarithromycin resistance.

Vaccines[51]

First evidence that protective immunization may, be a possibility came from Czinnand Nedrud in 1991. Urease was the early favourite vaccine candidate. Later both HspA and HspB protein were also shown to be immunogenic. Because of this and their apparant surface location, these proteins appeared as likely vaccine candidates. CagA and VacA are also being studied as vaccine candidates. Vaccine trails has been successful in animal models and studies are going on for a safe and effective vaccine.

Materials & Methods

MATERIALS AND METHODS

ETHICAL CONSIDERATION:

The study was conducted with the approval from the institutional Ethical Committee, GovernmentGeneral Hospital and Madras Medical College, Chennai-3.Permission to conduct the study was sought from the respective hospital authorities. Informed consent was obtained from the patients before the enrolment into the study.

PLACE OF STUDY:

Institute of Microbiology , Department of Medical Gastroenterology and Institute of Pathology, Rajiv Gandhi Government General hospital, Madras Medical College, Chennai-3.

STUDY PERIOD:

One Year[March 2017 ─February 2018]

STUDY TYPE:

Cross ─Sectional Study

SAMPLE SIZE:

120 Samples

STUDY POPULATION:

The isolates were obtained from gastric biopsy samples collected from peptic ulcer disease patients in Institute of Medical Gastroenterology, Rajiv Gandhi Government General Hospital, Chennai – 3.

INCLUSION CRITERIA:

- Adults > 18 years
- Patients with presenting with clinical history of Gastric ulcer, Duodenal ulcer ,Antral gastritis and Gastric carcinoma.

EXCLUSION CRITERIA:

Patients who were on antibiotics, proton pump inhibitors or Helicobacter pylori therapy within 1 month prior to this study.

MATERIALS:

- Endoscopy guided multiple Gastric biopsy samples collected from patients with peptic ulcer disease.
- 3 ml of venous blood collected under aseptic precautions.

METHODS:

1.Biopsy material would be subjected to

- Rapid urease test
- Histopathological Examination

- Culture inoculation which would be done on
  - Selective media - SKIRROW'S MEDIA
  - Non Selective media - Chocolate agar

2. Serum sample would be subjected to IgG Antibody assay by ELISA.

SPECIMEN COLLECTION AND TRANSPORT

Patients fasted overnight before endoscopy. Endoscopy was done using fiber optic endoscope. The endoscope and the biopsy forceps were rinsed thoroughly with water and soaked in 2% glutaraldehyde for 20 minutes and were thoroughly rinsed with sterile normal saline just before the collection of specimen.

Six biopsy samples were taken from the antrum (2 cm from the pylorus) and were transferred to respective sterile leak proof container. One sample was kept in commercial RUT kit after placing one drop of normal saline. This is performed at the time of gastroscopy.

There is colour change from yellow to red with in 5 minutes indicates positive reaction. Three specimens were tranported in normal saline, one is for culture and another two for PCR which should be kept in deep freezer. One is crushed between two frosted glassslides and used for gram stain and Giemsa stain. The last bit of sample was stored in formalin for histopathological examination.

The specimens for culture were transported in ice to the laboratory and were inoculated on the culture media without delay.

Blood:

3ml of venous blood was collected under aseptic conditions; serum was separated and stored at -20°C for further processing.

PROCESSING OF SPECIMENS;

Rapid urease test

An antral biopsy tissue was kept in commercial RUT kit after placing one drop of normal saline. This is performed at the time of gastroscopy. There is colour change from yellow to red colour indicates positive reaction.

CULTURE

Biopsy tissue was crushed between two sterile slides and the minced tissue was inoculated onto freshly prepared 5% defibrinated sheep blood and Skirrows supplement (selective media) and chocolate agar (non selective media) . The plates were incubated at 37°C in a candle jar . The plates were examined for bacterial growth from 3 to 7 days.

Characteristic small, translucent circular colonies were confirmed by gram stain, catalase,oxidase and urease. They were subcultured into chocolate agar and skirrow‧s supplement (Vancomycin 10mg, Polymyxin B 2500 IU and Trimethoprim 5mg) till no growth was obtained.

Cofirmatory tests for suspected colonies

1. **Gram stain;**

   Gram negative curved bacilli were seen.

2. **Oxidase test;**

   The suspected colony was streaked on the surface of oxidase strip containing 1% tetramethyl paraphenylene diaminedihydrochloride. An intense purple colour developed within 5 seconds and was recorded as positive. Positive and negativecontrols were used.

3. **Urease test;**

   In commercial kit, colour change from yellow to red colour indicates positive reaction.

4. **Catalase test;**

   The suspected colony was introduced with a class rod into 3% Hydrogen peroxide is taken in a clean test tube with Positive and negative controls. Immediate production of gas bubbles was noted as Positive.

CRUSH CYTOLOGY

Another biopsy tissue was crushed between the two sterile glass slides and the minced tissue was used to make smears.

GRAM STAIN

- One of the slide was air dried and heat fixed.
- The slide was covered with methyl violet for one minute, excess stain was poured off (primary stain)
- Grams iodine was added and washed after one minute (mordant).
- This was followed by acetone for 2 to 3 seconds(decolouriser).
- The acetone was washed and the slide was couterstained with dilute carbol fuchsin for 1 minute (counter stain), washed with water, blotted dry and observed under oil immersion objective.
- Helicobacter pylori appeared as gram negative spiral bacilli.

GIEMSA STAIN

- The other slide was air dried and fixed with methanol for 3 minutes.
- 2 to 3 drops of undiluted Giemsa stain was added and kept for 5 minutes.
- The smear was then washed with water, blotted dry and seen under oil immersion.
- The organism appeared deep purple with the typical gull wing shaped bacilli.

HISTOPATHOLOGY

One specimen was fixed in 10% formalin, paraffin sections were made and stained with Haematoxylin and Eosin (H&E) and examined for Helicobacter pylori.

SEROLOGY;

The serological detection ofIgG antibodies to cellular components of Helicobacter pylori was done using CALBIOTECH IgG ELISA KIT.

PRINCIPLE

- Patient's serum when added to wells coated with purified Hp antigen, H.pylori specific IgG antibody, if present, formed antigen-antibody complex which led to spectrometric reactions on subsequent addition of enzyme conjugate and substrateunder suitable incubation conditions.

- The intensity of spectrometric reactions as determined by the spectrophotometry was proportional to the amount of IgG specific antibody in the sample.

- The assays were validated as per the Quality control criteria of the Kit insert.

- The results were calculated as Antibody index and were interpreted to be Negative, Borderline and Positive based on the Antibody Index.Serology was done for 120 cases with gastroduodenal symptoms. 1 Positive control, 1 negative control and 2 calibrated standards were available in the kit.

PROCEDURE

Bring all the specimens and kit reagents to room temperature and gently mix.

1. Place the desired number of coated strips into the holder.
2. 1 Positive control, 1 negative control and 2 calibrated standards ready to use. Prepare 1: 21 dilution of test samples by adding 10µl of the sample to 200µl of sample diluents. Mix well.
3. Dispense 100µl of diluted sera, calibrator and controls into the appropriate wells. For the reagent blank, dispense 100µl sample diluents in 1A well position. Tap the holder to remove air bubbles from the liquid and mix well. Incubate for 20 minutes at roomtemperature.
4. Remove liquid from all wells. Wash wells three times with 300µl of 1X wash buffer. Blot on absorbance paper or paper towel.
5. Dispense 100µl of enzyme conjugate to each well and incubate for 20 minutes at room temperature.
6. Remove enzyme conjugate from all wells. Wash wells three times with 300µl of 1X wash buffer . Blot on absorbance paper or paper towel.

7. Dispense 100µl of TMB (tetra methyl benzidine) substrate and incubate for 10 minutes at room temperature. Add 100µl of stop solution.
8. Read O.D at 450 nm using ELISA reader within 15 minutes. A dual wavelength is recommended with reference filter of 600- 650 nm.

Calculation of results

The optical density of each calibrator was plotted against its concentration and calculate the cut off value by multiplying calibrator OD and calibrator factor (CF). Calculatethe antibody index of each determination by dividing the O.D. value of each sample by cutoff value.

Interpretation of Antibody index:

<0.9	-	No detectable Antibody
0.9 -1.1	-	Borderline Positive.
>1.1	-	Detectable Antibody

POLYMERASE CHAIN REACTION (PCR)

PCR was developed by Kary B Mullis in 1983.

PCR is a technique in molecular biology used to amplify a single or few copies of a piece of DNA to generate millions of copies of particular DNA sequence.

DIAGNOSIS OF H.pylori INFECTION BY PCR

In this study PCR was used to detect Helicobacter pylori by using primers specific for 16s rRNA gene. This gene is highly specific target for amplification and has been previously usedfor reclassification of the organism[61]. 16s rRNA is targeted to confirm H.pylori infection and positive amplification of H.pylori specific DNA may be considered as a direct evidence of the presence of pathogen[28].

PRINCIPLE OF PCR

PCR technique copies the target DNA by performing repeated cycles each containing the following three main steps.

1. DNA extraction from the organism /samples.
2. Amplification of extracted DNA
3. Gel electrophoresis of amplified products

1. DNA extraction from the organism /samples.

50 gastric biopsy samples were randomly selected for PCR study.samples were processed for PCR detection for16s rRNA of H.pylori. QIAGEN H.pylori detection kit was used.

- 25mg of gastric biopsy tissue (add 80µl PBS & minse with mortar & pessel if necessary)
- Transfer to 1.5ml tube. Add 60µl ATL buffer

- Add 20µl of proteinase K, vortex, then incubate at 56°C until the tissue is completely lysed. Thermomixer with rocking for 1-3 hours.
- Add 200µl AL buffer. Pulse vortex for 15 seconds and incubate at 70°C for 10 min.
- Add 200µl ethanol (100%); pulse vortex. Centrifuge briefly
- Transfer to mini spin column and close
- Centrifuge at 8000rpm for 1 minute
- Discard filtrate, transfer spin column into fresh 2ml collection tube
- Add 500µl AW1; centrifuge at 8000rpm for 1 minute.
- Discard the filtrate tube.
- Add 500µl AW2 buffer; centrifuge at 14000rpm for 3 minutes. Discard filtrate band and transfer spin column to new 1.5 ml tube.
- Add 200µl AE buffer, incubate at room temperature for 5 minutes. Centrifuge at 8000rpm for 1 minute. This step repeated 2 times for high yield of DNA (6µg of DNA/ 200µl)
- Final yield concentration of DNA is 30ng/µl.
- The extracted DNA sample was stored at -20°C for further processing.

COMPONENTS OF PCR

The primer was derived from the region of the 16s rRNA.

Forward primer - 5' GAGAATGAGATGAAACTCACCC 3'

Reverse primer - 5' TTGTCTGCTTGTCTATCAACC 3'

Components	**Final concentration of reagents**	**Quantity of reagents**
Template DNA	**5 – 100ng**	**5µl**
dNTP 2.5mM		
Buffer (10X)		
Forward primer		
Reverse primer		
Taq. Polymearse (GO Taq)		
Mgcl2		
Distilled water		25µl
Total volume		50µl

2. Amplification of extracted DNA

The PCR tubes are mixed well and kept in the thermocycler and the target DNA were amplified as given in the table below;

Procedure	Temperature	Time in minutes	Cycles
Initial Denaturation	95°C	5	1
Denaturation	94°C	1	35
Annealing	55°C	1	
Elongation	72°C	1	
Final Elongation	72°C	5	1

The amplified PCR products were stored at 4°C until electrophoresis.

3. Gel electrophoresis of amplified product

The amplified DNA was electrophoretically migrated according to their molecular size by performing horizontal 2.5% agarose gel electrophoresis. The amplified DNA formed a clear bands which can be visualised under UV light.

Detection of Amplified product

The amplified products detected by using fluorescent dyes like Ethium bromide or Syber green.

Molecular weight of the specified product is compared with standard molecular weight of restriction endonuclease digest of phage genome with differing molecular weight fragments

Interpretation;

A 294bp corresponds to 16s rRNA genes specific oligo - nucleotides.

Results

RESULTS

TABLE - 1; Demographic Profile of study Population(n=120)

AGE	MALE	FEMALE	TOTAL	PERCENTAGE
20 – 29	6	2	8	6.6
30 – 39	33	10	43	35.8
40 – 49	19	12	31	25.8
50 – 59	15	3	18	15
>60	16	4	20	16.6
TOTAL	89	31	120	100

Male preponderance (74.1%) is more in third decade of life

CHART – 1

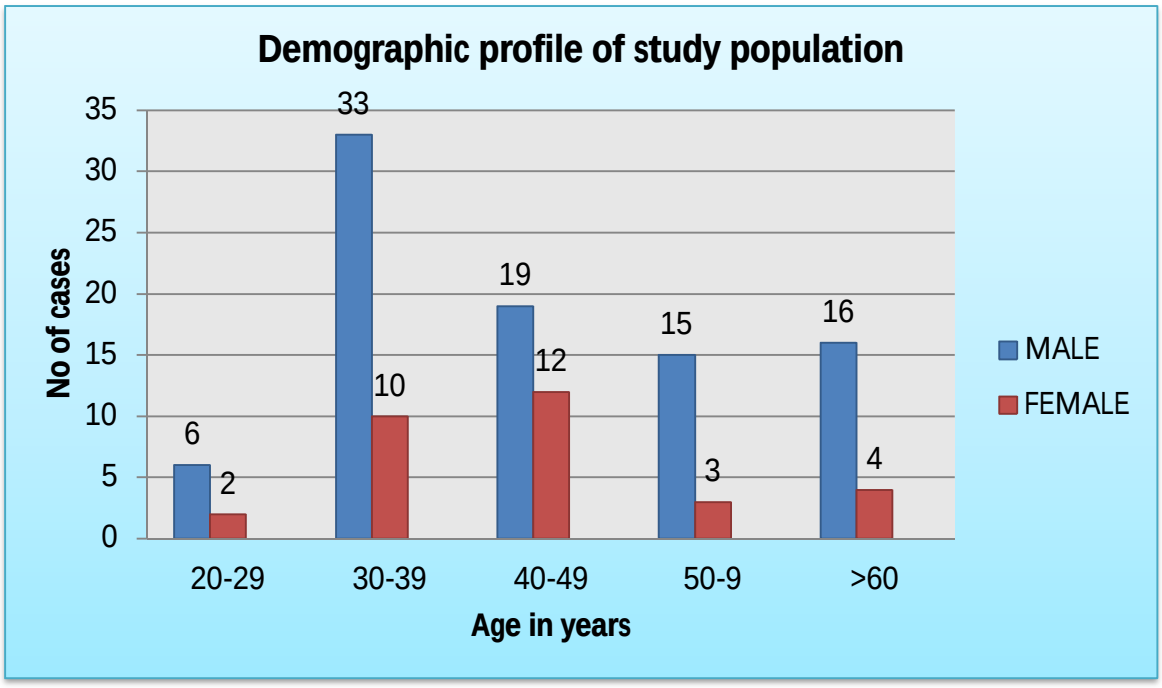
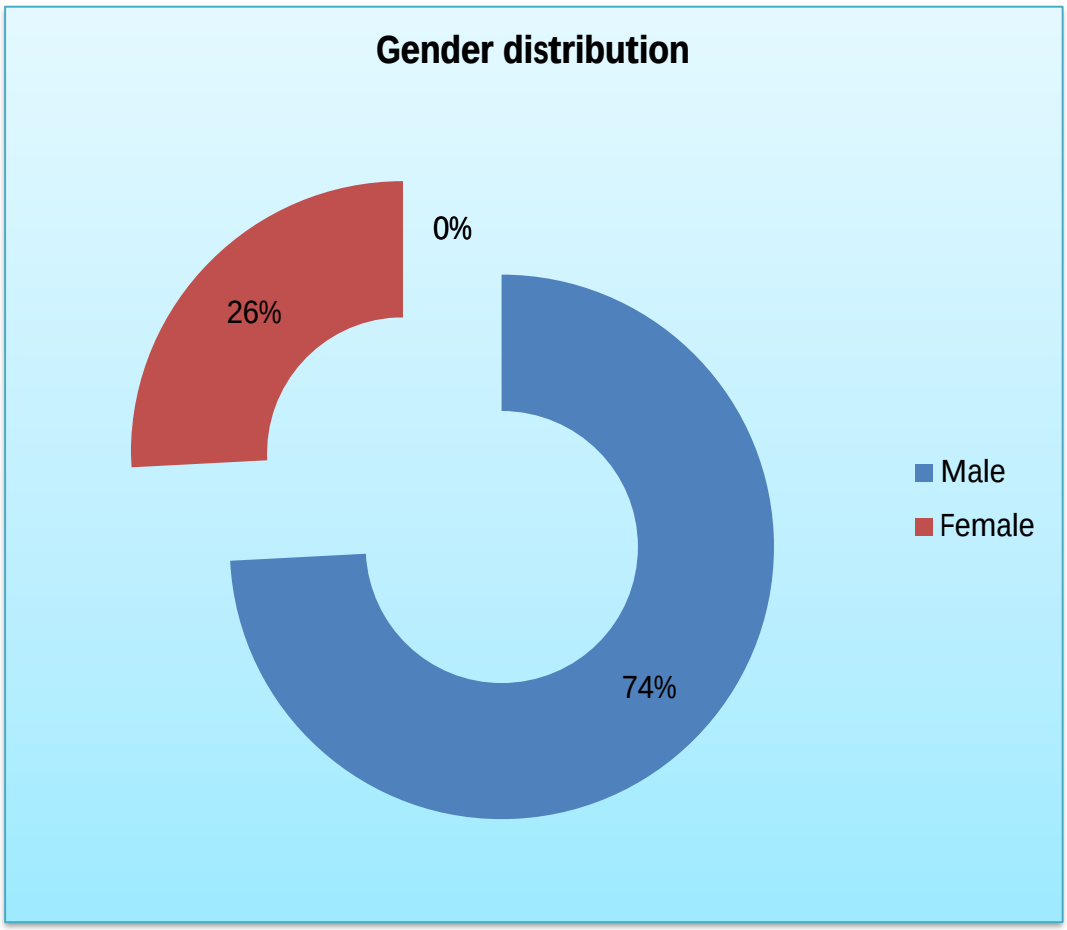


CHART - 2

Sex wise distribution of study Population (n=120)



Among the 120 study population, 89 patients were males, 31 patients were females. Male preponderance was high among the study population

TABLE - 2

	MALE	FEMALE	TOTAL
NO. OF CASES	89	31	120
PERCENTAGE	74.1	25.8	100

TABLE – 3

Symptoms and Gender distribution in relation to study population (n=120)

Symptoms	Gastric carcinoma (n=29)			PUD (n=91)		
	Male	**Female**	**%**	**Male**	**Female**	**%**
Epigastric pain	24	2	89	59	19	86
Vomiting	17	2	65	46	12	64
Dyspepsia	6	5	37.5	59	29	80
Appetite loss	13	3	55	12	9	22
Weight loss	14	4	62	15	18	35
Haemetemesis	6	1	24	7	4	12
Melena	5	5	31	3	2	5

Epigastric pain was the most common symptom (89%) among the study population

CHART - 3

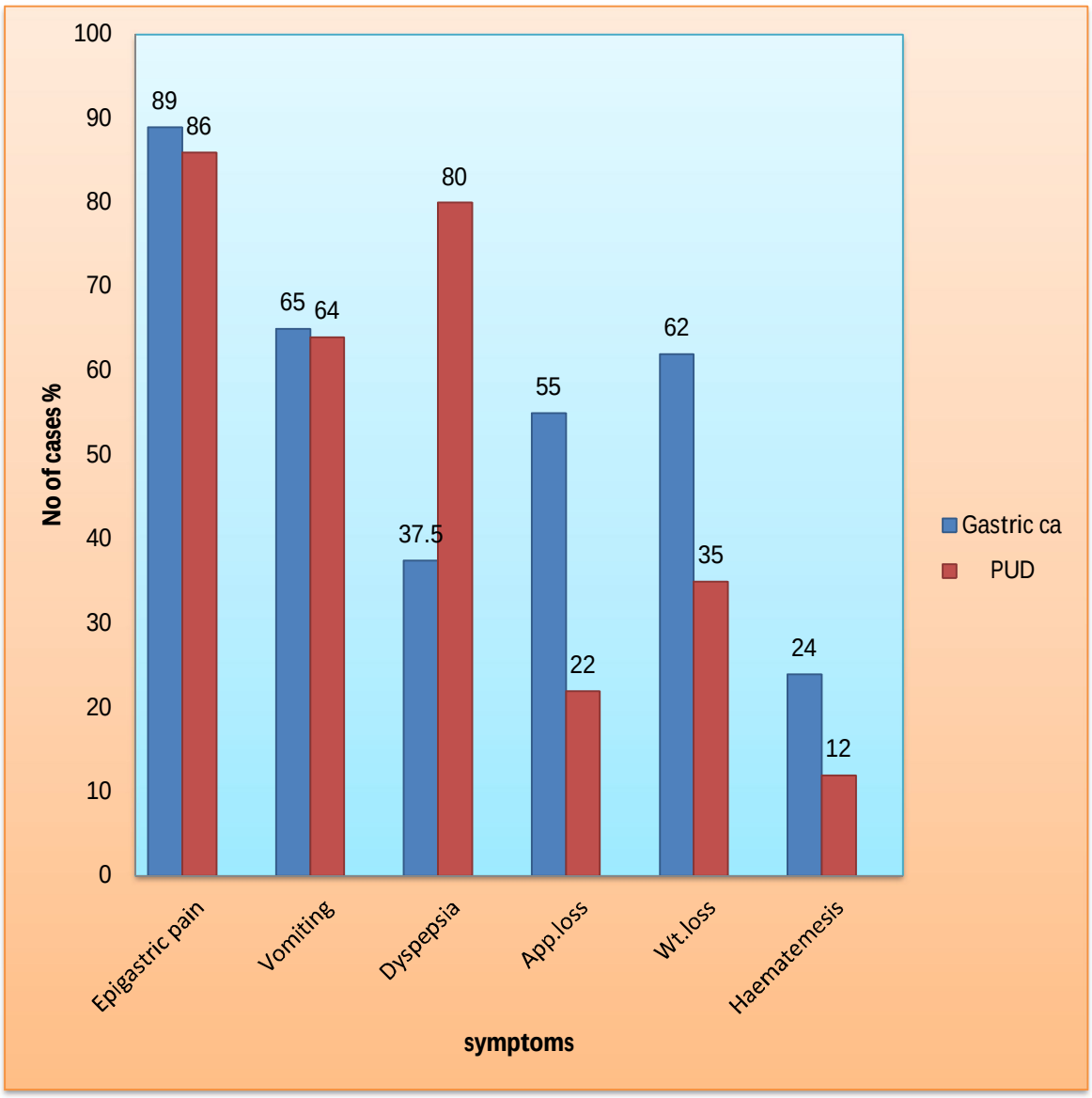

100
90
80
70
60
50
40
30
20
10
0
No of cases %
89
86
65
64
37.5
80
55
22
62
35
24
12
Epigastric pain
Vomiting
Dyspepsia
App.loss
Wt.loss
Haematemesis
symptoms
Gastric ca
PUD

TABLE - 4

Categorization of study population based on Endoscopic diagnosis(n=120)

Endoscopic diagnosis	Total	Percentage
Duodenal ulcer	46	38
Gastritis	30	25
Gastric ulcer	15	13
Gastric carcinoma	29	24
Total	120	100

Duodenal ulcer was the most common (38%) endoscopic diagnosis among the study population

CHART –4

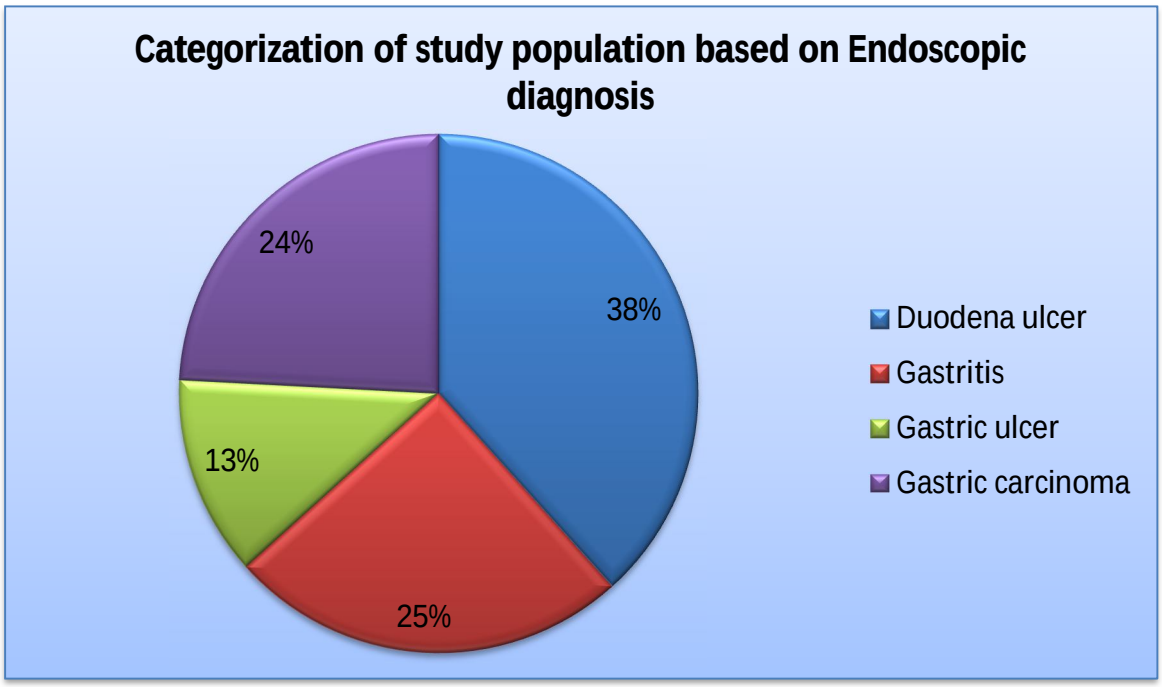


TABLE – 5

Rapid urease Test Positivity among Endoscopically diagnosed gastrodudenal diseases (n=120)

Endoscopic diagnosis	Total	Positive	Percentage
Duodenal ulcer	46	25	54.3
Gastritis	30	15	50
Gastric ulcer	15	10	66
Gastric carcinoma	29	15	51.7
Total	120	65	54

The overall positivity of RUT was 54 % . but if the gastroduodenal diseases are analysed individually, RUT was positive in 54.3% of patients with duodenal ulcer disease.

CHART – 5

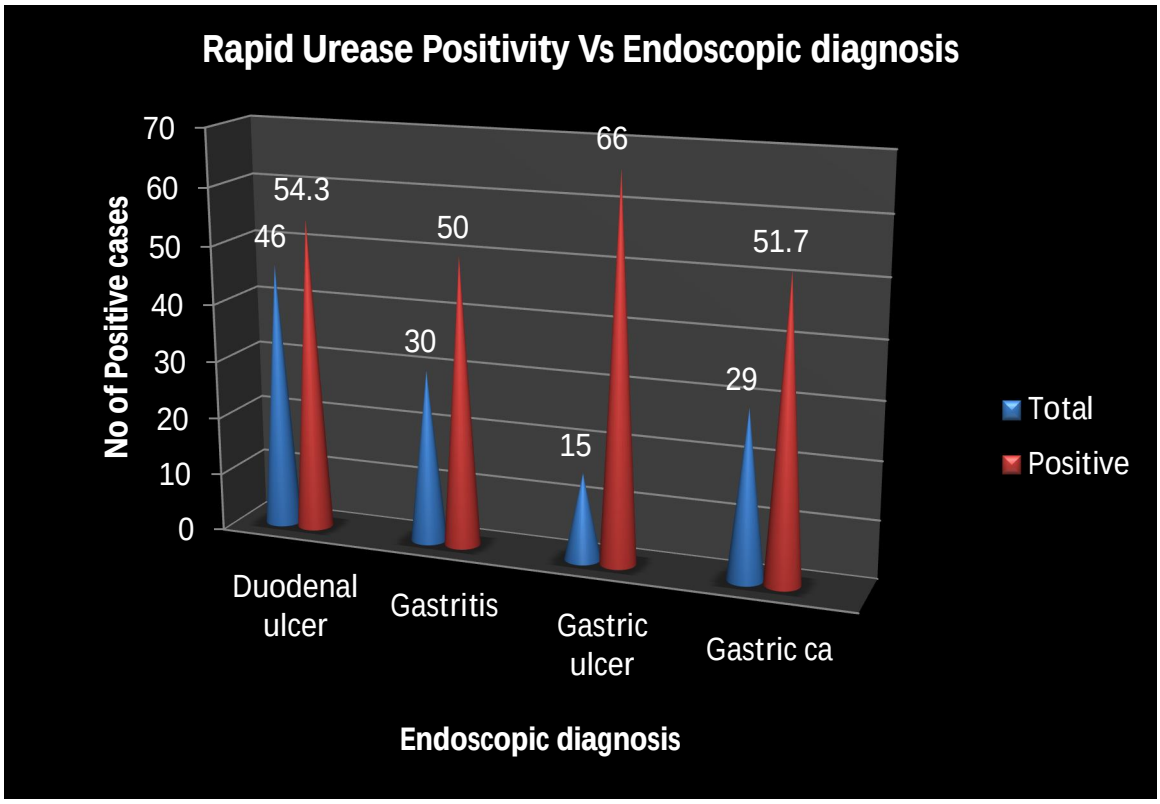


TABLE – 6

Positivity of Gram negative bacilli by Gram stain (n=120)

Endoscopic diagnosis	Total	Positive	Percentage
Duodenal ulcer	46	12	28
Gastritis	30	6	14
Gastric ulcer	15	12	28
Gastric carcinoma	29	13	30
Total	120	43	35.8

35.8 % of Gram negative bacilli detected by Gram stain

CHART- 6

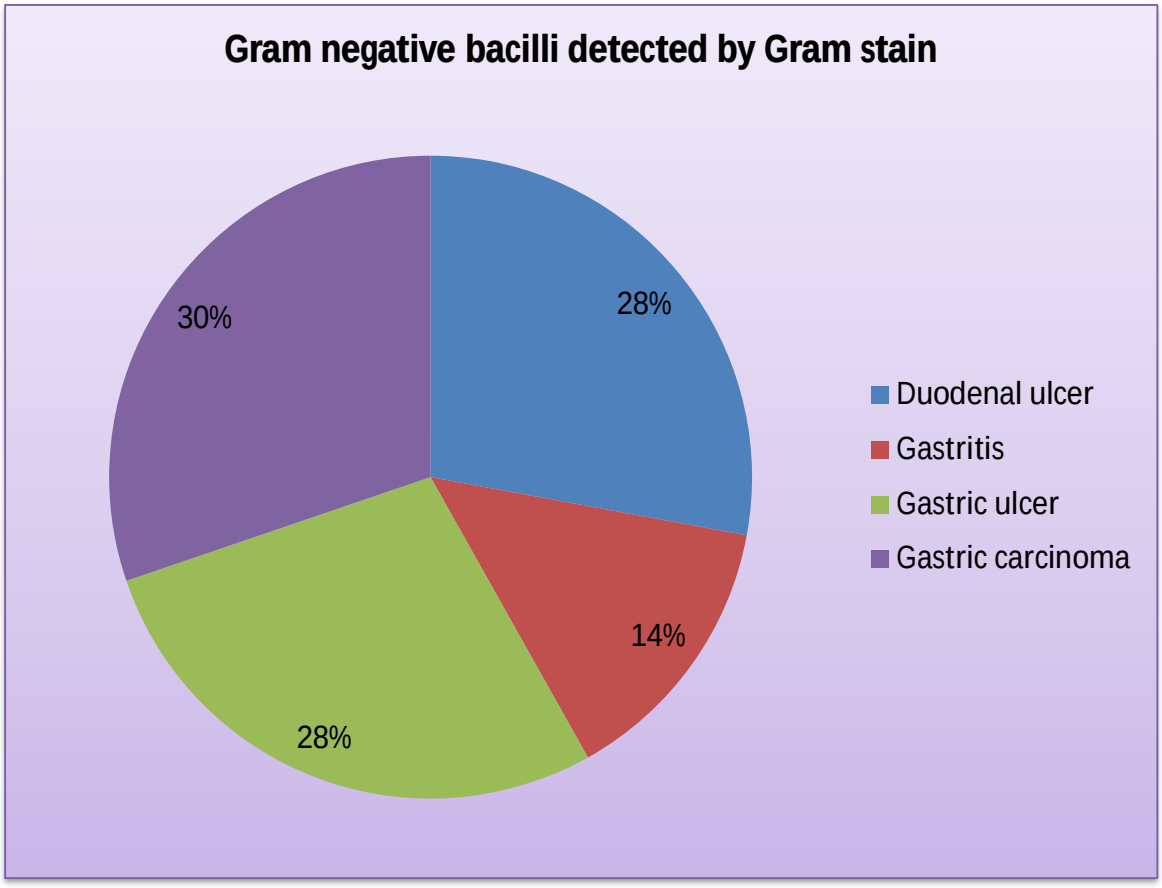


TABLE – 7

IgG ELISA among Endoscopically diagnosed gastroduodenal diseases (n=120)

Endoscopic diagnosis	Total	IgG ELISA	Percentage
Duodenal ulcer	46	5	4.1
Gastritis	30	5	4.1
Gastric ulcer	15	5	4.1
Gastric carcinoma	29	18	15
Total	120	33	27.5

Seroprevalence in the study Population was 27.5%.

CHART- 7

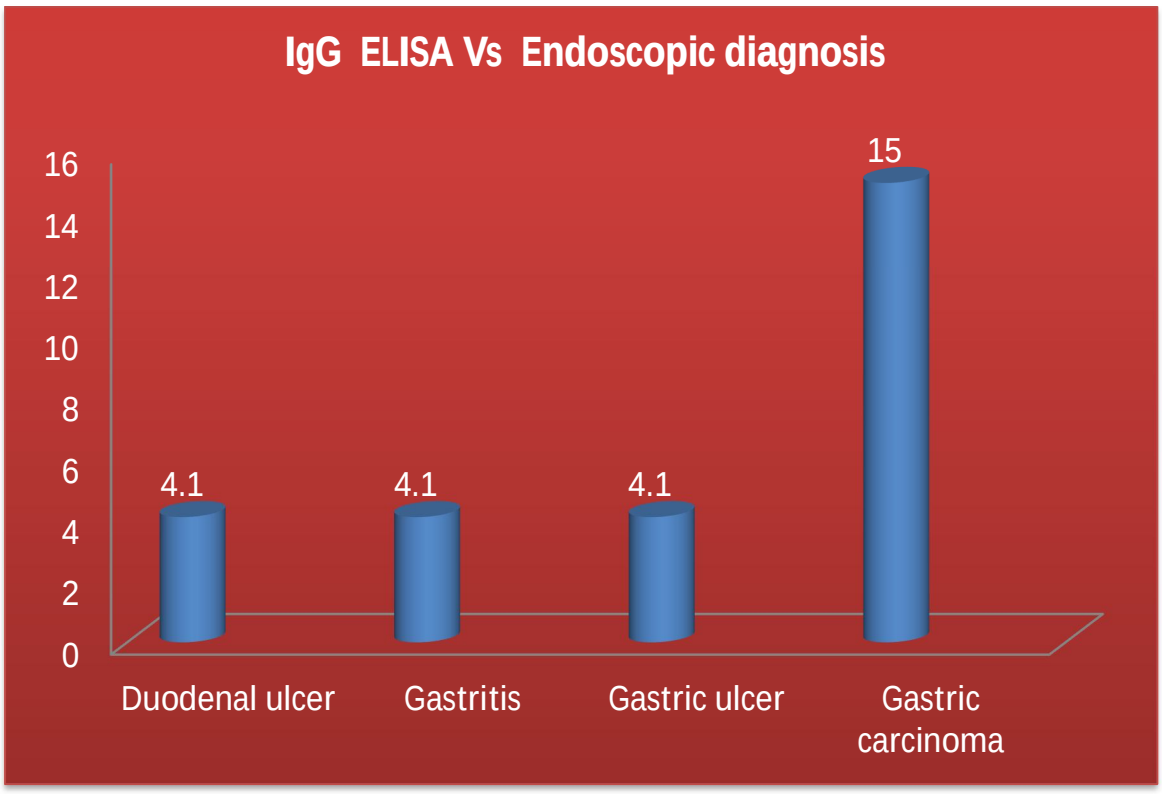


TABLE - 8

Comparision of RUT with H.pylori Positive by Gram stain, IgG and HPE (n=120)

Endoscopic diagnosis	No of cases	RUT	Gram stain	IgG ELISA	HPE
Duodenal ulcer	46	25	12	5	8
Gastritis	30	15	6	4	12
Gastric ulcer	15	10	12	5	6
Gastric carcinoma	29	15	13	15	45
Total	120	65	43	29	71

CHART – 8

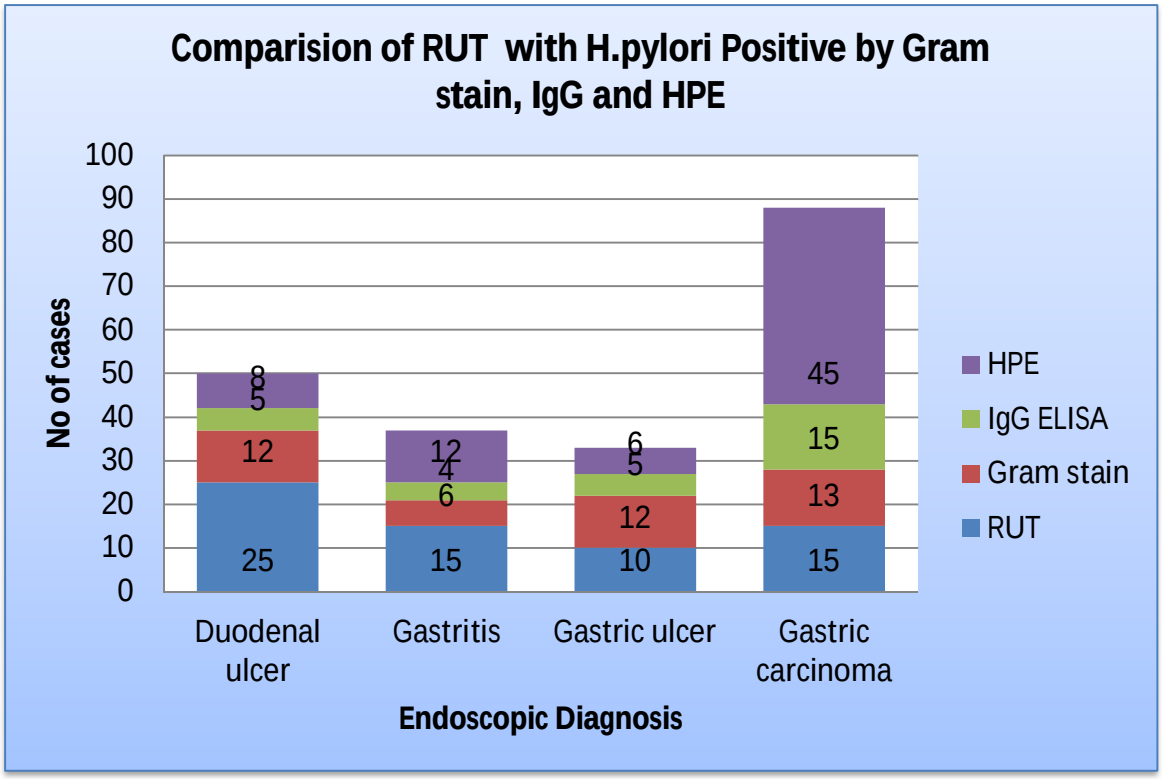


TABLE – 9

Correlation of HpIgG and HPE report (n=116)

	HpIgG Positive	HpIgG Negative
Malignant lesions	29 (25%)	32 (27.5%)
Non malignant lesions	0	55 (47.4%)

29 patients of histopathological finding of malignant lesions were positive for HpIgG

CHART – 9

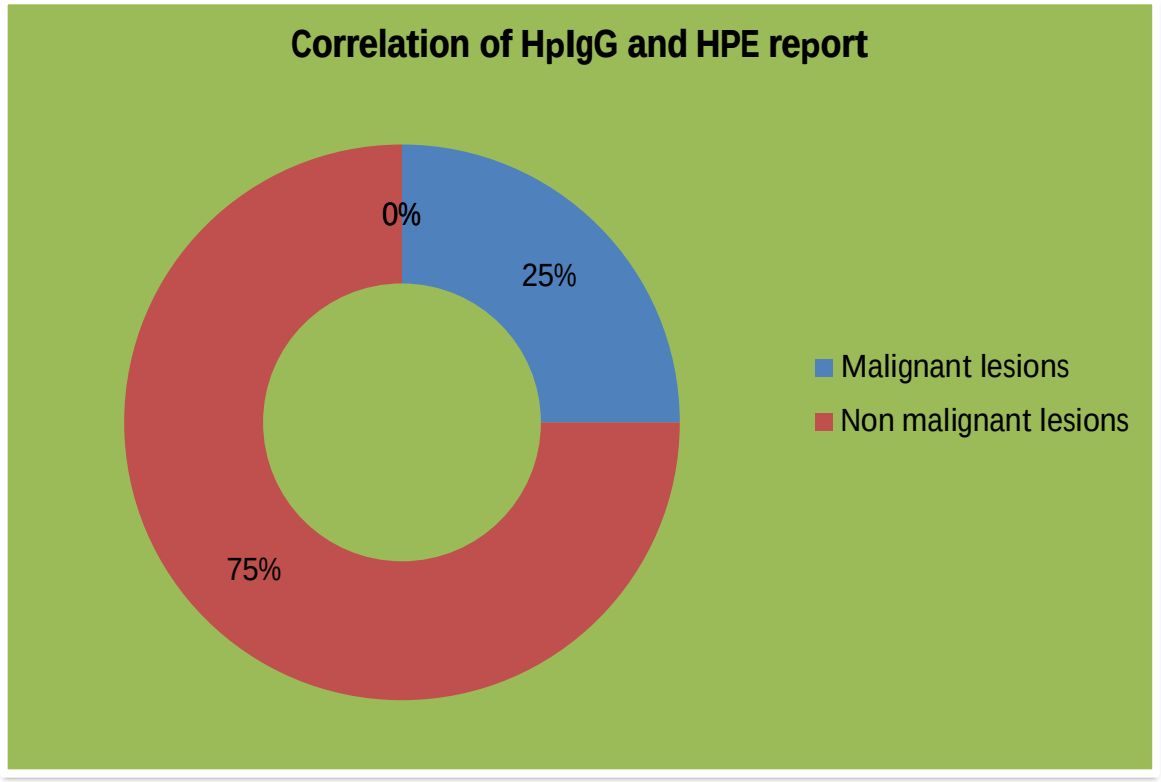


TABLE-10

Results of Rapid urease test in comparision with HPE reports

Clinical status	No of case	RUT Positive	RUT Negative
Malignant lesions	64	50	14
Non malignant lesions	56	15	41
Total	120	65	55

50 &15 patients were positive RUT in malignant &non malignant lesions respectively.

CHART -10

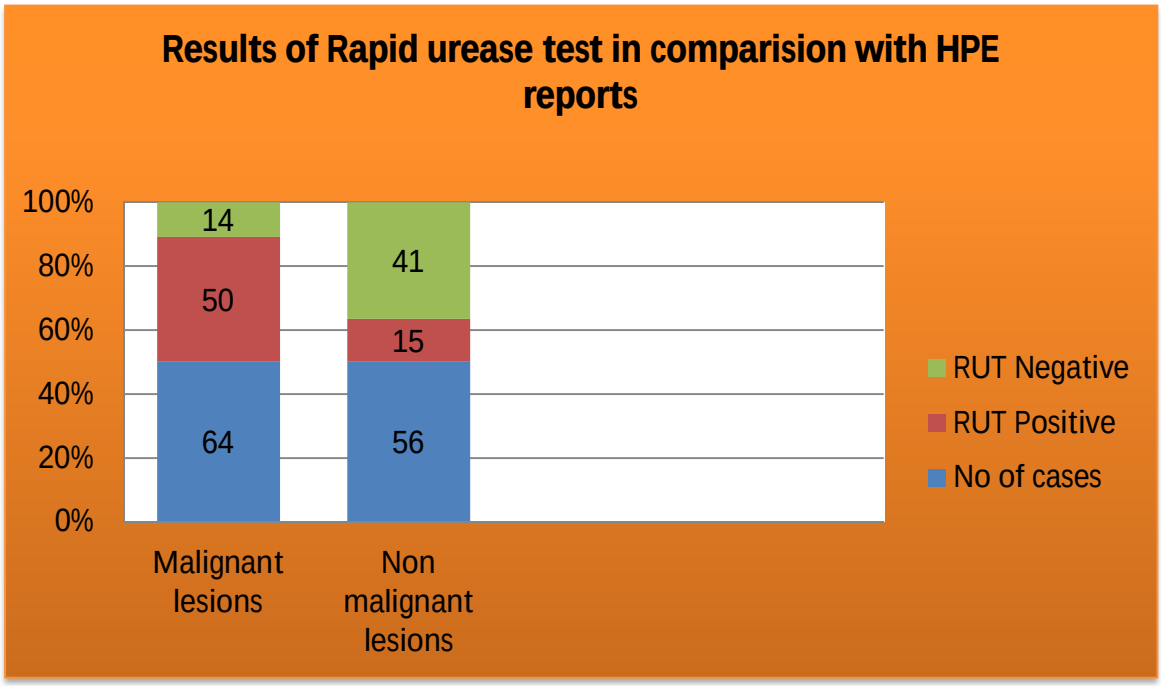


TABLE -11

Comparative evaluation of conventional methods and ELISA based IgG antibody detection

Type of Test	Patients (n=120)	
	Positive	Percentage
Rapid urease test	65	54
IgG ELISA	29	24.1
HPE	71	59.1

HPE findings correlates with more cases than other conventional methods

CHART – 11

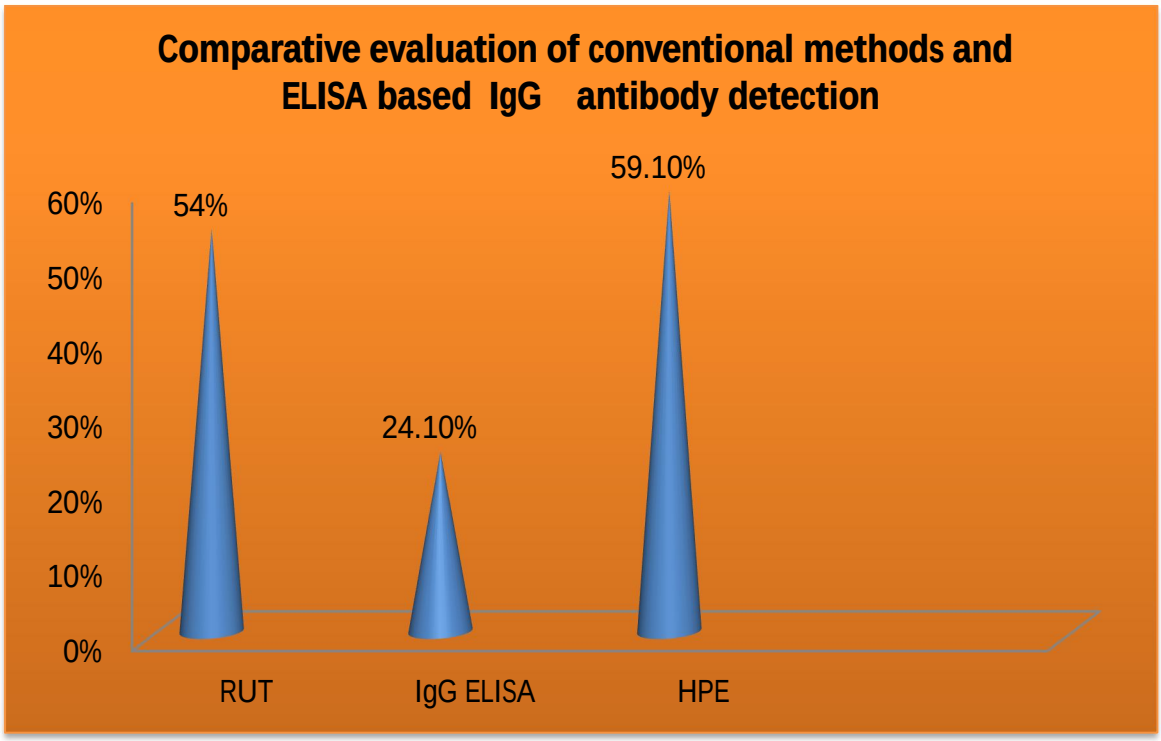


TABLE- 12

Correlation of HPE results with various diagnostic tests for H.pylori(n=50)

	RUT	Gram stain	HPE	PCR
Malignant lesions	28	27	33	18
Non malignant	7	5	10	5
Total	35	32	43	23

Hispathological finding highly correlates with RUT of malignant (28) and non malignant (7) lesions.

CHART – 12

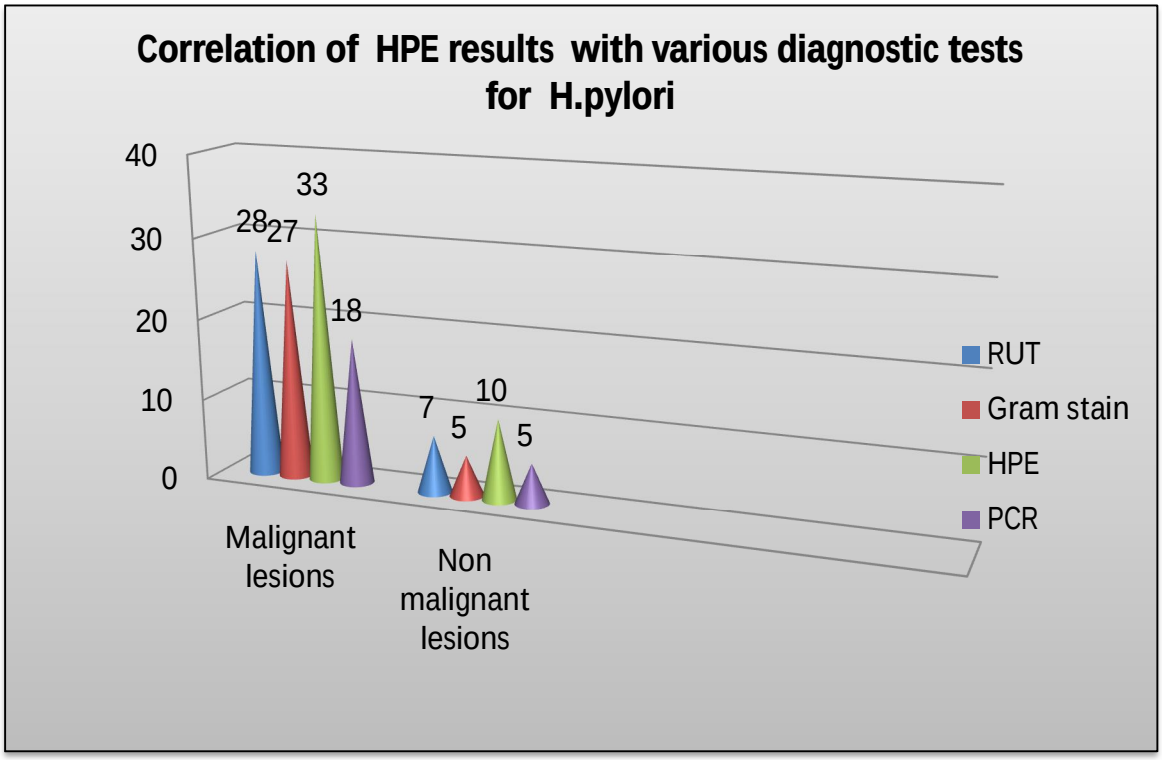


TABLE -13

Correlation of PCR with conventional test in 50 samples (n=50)

Test	Positive	Percentage
PCR	23	46
RUT	35	70
Gram stain	32	64
HPE	43	86
IgG ELISA	29	58

Histopathological findings consistent with 33 patients of gastric malignant lesions and 10 patients of non malignant lesions among randomly selected 50 samples.

CHART-13

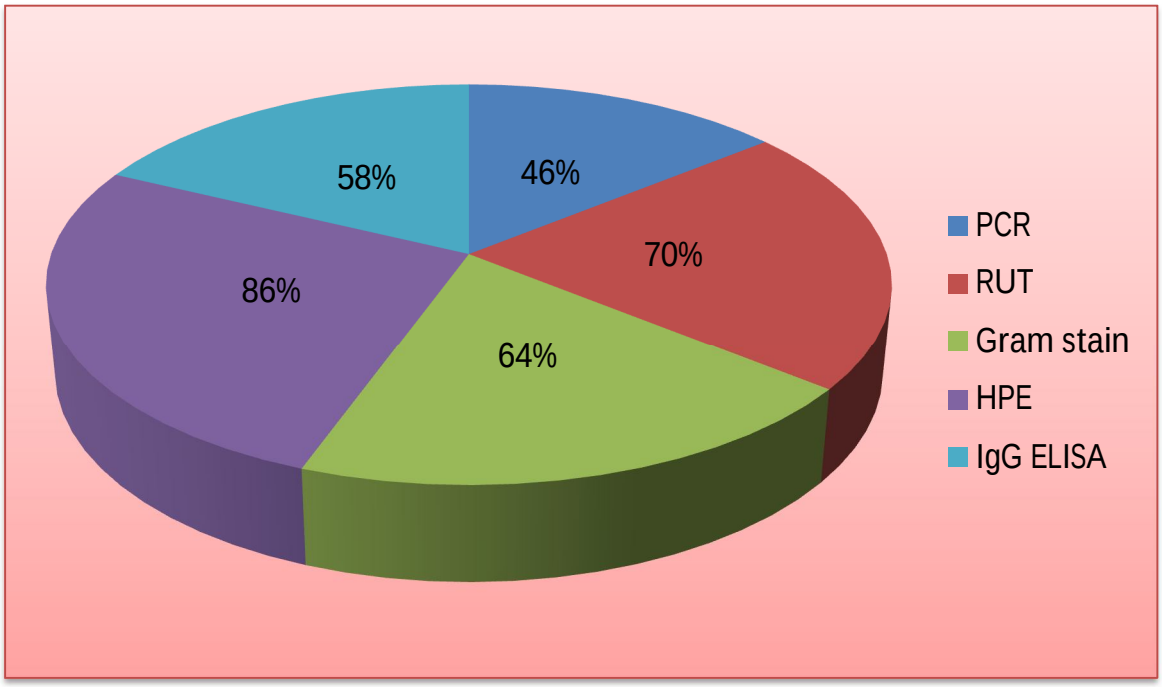


TABLE – 14

Correlation of PCR with HpIgG (n=50)

Name of Test	Positive	Percentage
PCR	23	46
HpIgG	29	58

HpIgG was found to be positivein 58% of cases, PCR positive in 46%of cases.

CHART – 14

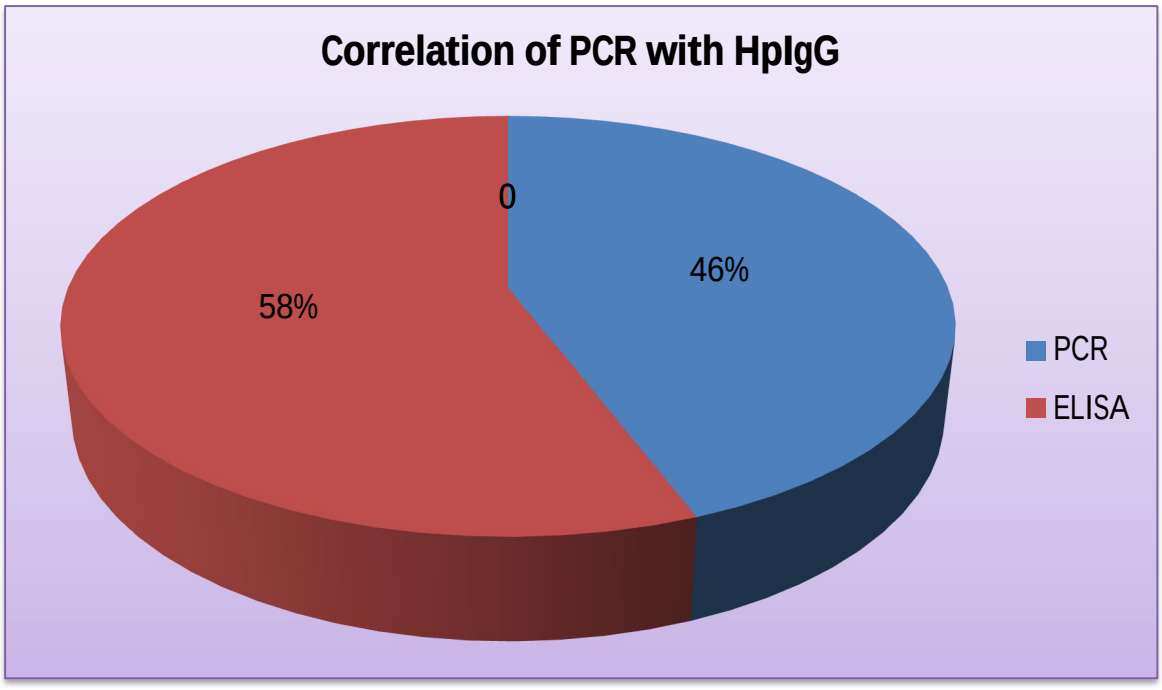


TABLE – 15

RUT Vs PCR (n= 50)

PCR	RUT Positive	RUT Negative
Positive	15	4
Negative	20	11

PCR was positive in 23 cases and RUT was positive in 35 cases among 50 patients of gastroduodenaldisease.PCR and RUT , both positive in 15 cases. Both were negative in 11 cases.

RUT was positive in 35 cases in which 28 were malignant lesions and 7 were non malignant lesions. PCR was positive in 23 cases which includes 18 were malignant lesions and 5 were non malignant lesions.

CHART – 15

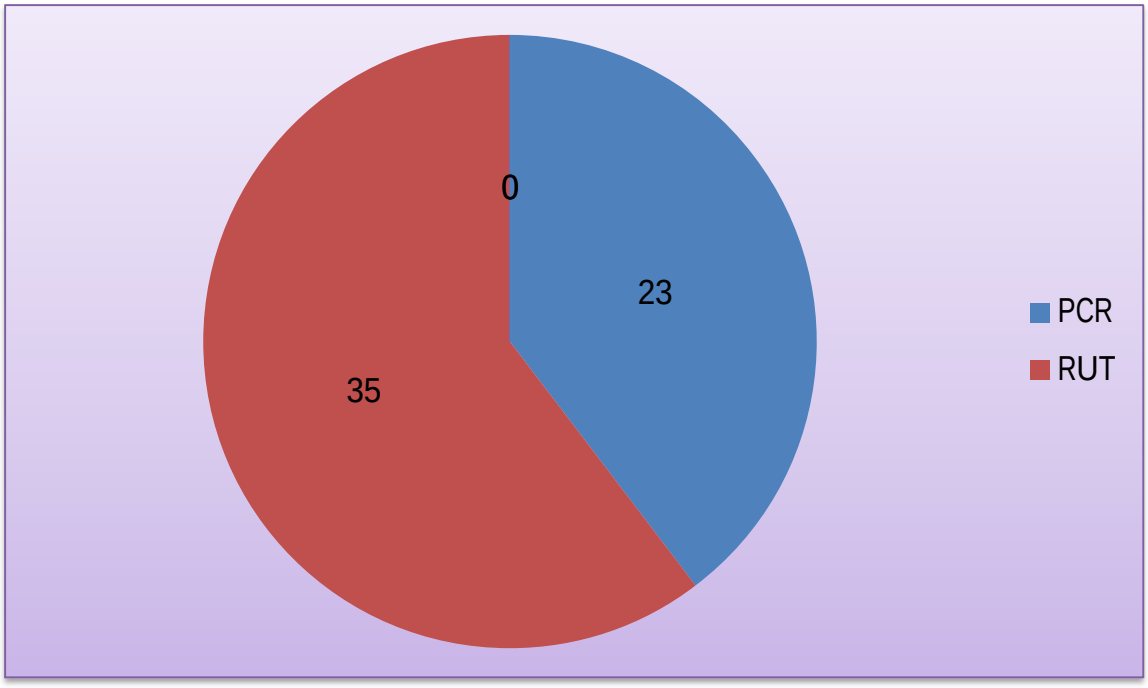
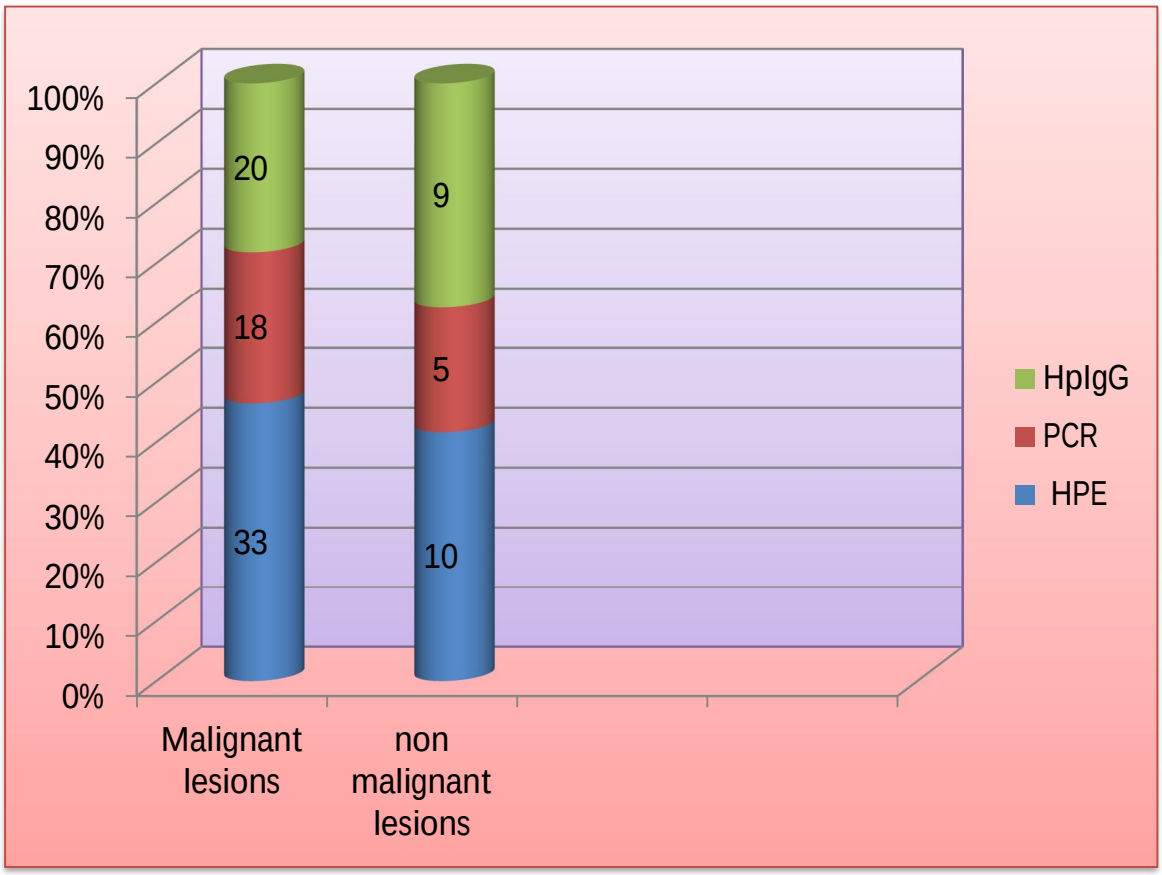


TABLE – 16

Correlation of PCR and HpIgG with HPE (n=50)

HPE	PCR for H.pylori		HpIgG	
	Positive	**Negative**	**Positive**	**Negative**
Malignantlesions	18	20	20	10
Non malignant lesions	5	7	9	11
Total	23	27	29	21

CHART – 16



Discussion

DISCUSSION

In this study, 120 patients with clinical diagnosis of gastroduodenal disease and endoscopic diagnosis of gastric lesions attending the gastroenterology department of Rajiv Gandhi Government General Hospital included endoscopy guided gastric biopsy samples were obtained from the patients which were subjected to Gram stain, Giemsa stain, Rapid urease test, histopathological examination, bacterial culture and PCR with appropriate controls to diagnose *H.pylori* infection. Blood samples were obtained from these patients for serological work up.

The results were analysed with SPSS (Statistical Package for Social Sciences) software in correlation with detailed clinical findings.

Duodenal ulcer was the most common Endoscopic diagnosis among the study population. In this study, there was a male prepondarance 89 (74.1 %) among the study population and the maximum number of patients with were in third decade of life.

In the present study, among the patients with peptic ulcer disaese , the Epigastric pain was the predominant symptom (86%) dyspepsia in 80%, vomiting 64% , loss of weight 22% , haematemesis in 12% and melena in 5% of cases. Similarly Epigastric pain was the predominant symptom among the patients with gastric carcinoma (89%) , followed by vomiting (65%). But in patients with gastric malignancies loss of weight (62%) and loss of appetite (55%) were

strikingly high when compared with the patients with non malignant lesions. Haematemesis in 24% , dyspepsia 37.5% and melena in 31% of the patients in the study group.

The endoscopic diagnosis of this study population revealed that duodenal ulcer accounted for 38%, suspected malinant lesions in 29% , gastritis in 25% and gastric ulcer in 13% .

All the 120 patients were subjected to Rapid urease test, Gram stain, Giemsa Stain ,histopathological examination, and bacterial culture . PCR was done for 50 Samples (randomly selected).

Among 120 gastric biopsy samples, the overall positivity of rapid urease test was 54% . But if the correlation between RUT and gastroduodenal diseases are analysed individually, 66% of patients with gastric ulcer were positive for RUT, 54.3% of patients with duodenal ulcer 51.7% of patients with gastric carcinoma (Table - 4). The findings of the present study correlates with the study done by Sivaprakash et al in which Rapid urease test was positive in 38.7% of patients with gastroduodenal disease[65] .

Among the various diagnostic methods to detect the presence of H.pylori in gastric biopsy samples, employed in this study, rapid urease test showed maximum positivity in 54% cases, HpIgG in 24% of cases .

H.pylori was not isolated from any of these samples and Hence , culture was not contributory in the present study. In the study conducted by Anjana et

al[66],[35],[11], in which 34 out of 47 cases were positive forRapid urease test and culture positivity is 17.6%.

This low isolation rate may be due to the patchy distribution of *H.pylori* in gastric mucosa, its fastidious nature, mucosal atrophy[63],[35], administration of antibiotics (for some other infections) and proton pump inhibitors.[7]

The direct gram stain showed gram negative bacilli about 35.8 % in which 17% of samples (21smears) revealed Gram negative bacilli morphologically consistent with H.pylori. These results were comparable with the study done by U. Arora et al[63] , RUT was positive in 72%,direct microscopy of Gram stain in 20% of cases and 28% of culture positive cases. All cases which were positive by rapid urease test , had significant IgG levels (76%) (Table - 6).

In this study histopathological examination was consistant with malignant findings for 71(59.1%)cases and 49(40.8%) cases of non malignant lesions. (Table – 11) This is compared with study conducted by Aarti et al[47] that showed histopathological findings highly correlated with clinical diagnosis of gstroduodenal disease.

Among the 120 patients included in the study, HpIgG antibodies were Positive in 33 (27.5%) patients ,which includes 4 patients with borderline Positive in and negative in 87 (72.5%) patients. Among the 33 patients with Positive HpIgG (Titre ≥ 1.1and range between 1.1 and 2.7), 32 (96%) were diagnosed to have gastric malignancies by histopathological examination and 1

patient with non malignant lesions. Among 87 patients who had HpIgG titre ≤0.9 (ie) negative, 32 (36.7%) of them were diagnosed to have malignant gastric lesions and 55(63.2%) of them were having non malignant lesions as with HPE report.

4 Patients who tested borderline positive for HpIgG, were analysed separately, 3 of them were diagnosed with malignant and 1 with non-malignant gastric lesions on histopathological examination.

In the study conducted by Ruud J.L. F. Loffeld et al in dept of gastroenterology[1], free university hospital , Netherland in Jan 2000, correlation of *H.pylori* IgG antbody titre with malignant and non malignant gastric lesions, *H.pylori* colonization was found to be a risk factor in 28% of patients with gastric malignancy. Among the 64 patients with malignant gastric lesions, 29 were sero positive for HpIgG (45.3%) . (Table - 9)

The prevalence of patients with *H.pylori* antibodies (HpIgG) was highest (80%) in the youngest age group (30-49 years) and, in contrast to the prevalence of *H pylori* antibodies in the general population, showed the study conducted by P Sipponenet al[20]. In all age groups the prevalence of *H pylori* antibodies was higher in the patients with gastric carcinoma than in the controls without gastric carcinoma.

In the PCR test, 23 cases were positive among 50 samples (randomly selected) which includes 18 cases of gastric carcinoma and 5 cases were peptic ulcer disease.

Among the 50 gastric biopsy samples from patients with gastroduodenal disease which were subjected to PCR 23(46%) were positive for H.pylori with band at 294bp. Among the positive PCR samples, RUT was positive in 15 (65.2%), Gram stain was positive in 17 (21%). PCR was negative in 27 patients amongest whom RUT was positive in 20 (40%) samples.

When the results of PCR are compared with serum HpIgG results in these 50 patients, 12 (24%) patients were positive by PCR as well as serum IgG, whereas 15(30%) patients were negative by both. 8 patients(16%) of gastric samples were positive for H.pylori gene by PCR in whom serum HpIgG were negative. In 13 patients (26%) HpIgG was positive but PCR was negative.

Histopathological examination consistent with PCR in 18 cases of malignant lesions and 5 cases of non malignant lesions among 50 study population. P value is 0.7297 ,Since the sample size was very low in tertiary care centre, difficult to attain the p value < 0.05.

Among the 71 patients with malignant gastric lesions, 29 were positive for HpIgG (40.8%). 54% were positive for H.pylori by RUT . Among the 29 patients with malignant lesions who were subjected to PCR, 18 (46%) were positive by PCR.

The study conducted by Parmar A et al[67] , HPE results showed 66.7% cases were Adenocarcinoma which is the commonest tumour of stomach. HPE was the important diagnoatic tool for malignancy. (Table – 12)

Hospital sirio – Libanes et al[18] study shows the strong correlation between the *H.pylori* infection detected by PCR, and the histological findings from gastric biopsies noteworthy. PCR was positive in 86% of moderate to marked gastritis cases, 67% of adenocarcinomas and 100% of MALT lymphomas.

Rapid urease test correlated with PCR hence, RUT was positive in 35 cases which includes 28 were malignant lesions and 7 were non malignant lesions. PCR was positive in 23 cases which includes 18 were malignant lesions and 5 were non malignant lesions. It shows the significant P value <0.024.

Jae – Sik Jeon et al [68] study showed that combination of RUT and PCR is a valuable diagnostic method and faster identification of antibitic resistance at the genetic level. The detection rate was 88.1% and 89.1% by RUT and *H.pylori* PCR respectively. RUT result is dependent on bacterial (at least 1X 10^5 copies) , and has high sensitivity >95%. Thus in cases of samples with low bacterial load need to be tested with PCR for reliable results.(Table – 15)

Limitations of the Study

LIMITATIONS OF THE STUDY

Since this is a single centered study with low sample size, the true prevalence of *H.pylori* infection could not be evaluated. Multi-centered studies with larger sample size are required to identify the overall prevalence of *H.pylori* infection in the community.

Since the gastric biopsy sample is very precious, little bit inconvenience for getting consent from patient for sample collection. Symptomatic patients were only subjected to endoscopic diagnosis, so there is possibility of missing asymptamatic *H.pylori* infection cases.

Molecular characterization was done only for the identification of *H.pylori* from gastric biopsy tissue. There are various genes responsible for detection of *H.pylori* infection, which were not done in this study.

The combination tests of RUT and PCR should be performed for rapid and appropriate diagnosis of *H.pylori* and detection of antibiotic resistance. Hence, this is not possible to all cases due to expensive diagnostic method.

Summary

SUMMARY

- The majority of the patients in the study population (120) were in the age group of 30-39 years (35.8%).
- A male preponderance (74.1%)was noted among the study population.
- Epigastric pain was the most common symptom in both gastric carcinoma and peptic ulcer disease.
- Duodenal ulcer was the Predominant endoscopic finding observed in the study.
- Rapid urease test was positive in 54% of the samples. RUT is a screening method to detect *H.pylori* infection with good sensitivity.
- Gram stain showed Gram negative bacilli in 35.8% of the cases.
- The seroprevalence of (HpIgG) among the study population was 27.5%.
- On HPE 59.1% of Gastroduodenal lesions were observed to be malignant and 40.9% to be nonmalignant.
- Among the malignant lesions as the HPE report H.pylori infection was detected in 21% of patients by RUT (n=120), 62% by PCR (n=50), HpIgG seroprevalence among the study population was 27.5%. Among the 71 patients with malignant lesions 32(45%) patients were positive for HpIgG by ELISA and 39(54.9%) were negative for HpIgG.
- .

- Among the 49 patients with non malignant lesions RUT was positive in 50(41.6%) of patients whereas PCR was positive in 10% of non malignant lesions.
- Among the 49 patients with non malignant lesions 10 (20.4%) patients tested positive for HpIgG.
- PCR was positive in 12% of patients with Negative RUT and 20% of patients who tested negative for HpIgG .

Conclusion

CONCLUSION

- A high preponderance of *H.pylori* infection was noted in patients with duodenal ulcer.

- The simple and inexpensive Rapid urease test detected maximum number of positive cases among the conventional tests on endoscopy room. Hence, it is a valuable screening test which could be used as an adjunct to endoscopy.

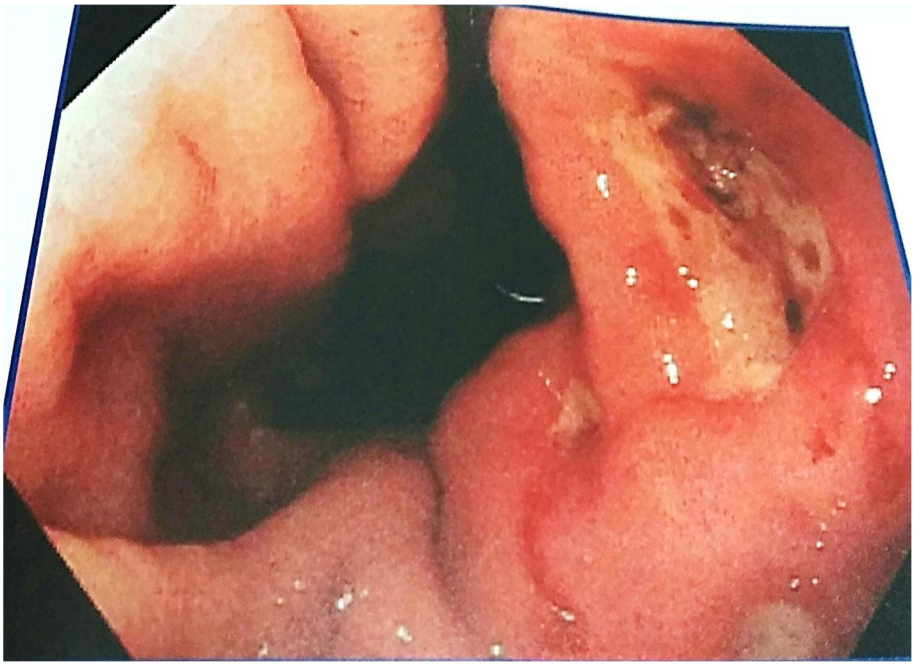
- HpIgG antibody assays could be employed as a useful screening assay with stringent quality control measures in place for the estimation of seroprevalence of *Helicobacter pylori* among the high risk population. However subsequent serum samples are required to follow up the severity of the disease, and a larger sample size for a point prevalence study.

- Isolation of organisms has varied sensitivity (2% to 26%) which are restricted only to reference laboratories.
- Hence PCR is a better alternative to culture,to diagnose H.pylori infection as Nucleic acid amplification methods are more sensitive,if appropriate primer probes are utilized.

- The combination test of rapid urease test with PCR serves as a rapid and appropriate diagnostic method for *H.pylori* infection.
- When compared with uninfected subjects ,persons infected with H.pylori including those who had seroconverted were at high risk of developing gastric carcinoma.
- As H.pylori infection is a chronic illness with added risk factors they play a definitive role in gastric carcinogenesis. Hence diagnosis of H.pylori infection should be routinely done in high risk population with the determination of their serological status , to plan for successful eradication of the infection and hence , reducing the risk of carcinogenesis.

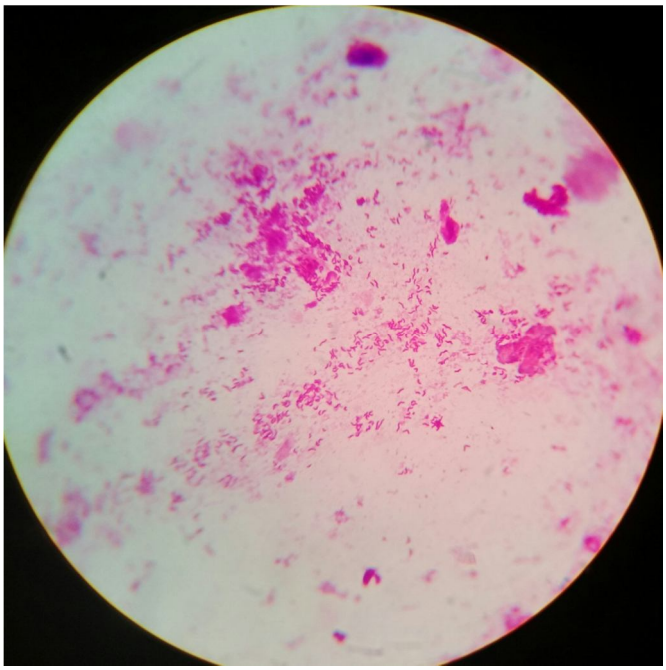
Colour Plates

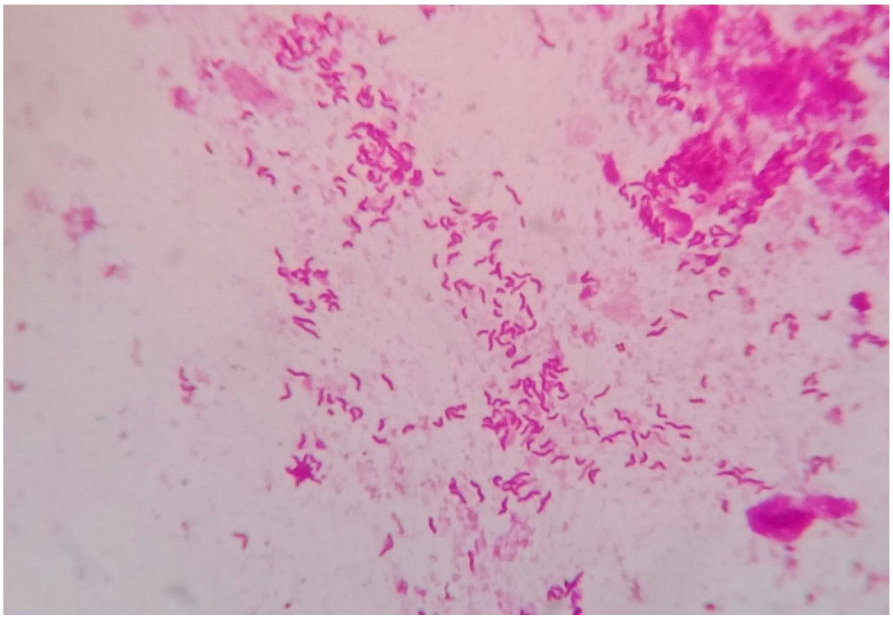
1. A CASE OF GASTRIC ULCER



2. A CASE OF GASTRIC CARCINOMA

3. GRAM STAIN OF H.pylori INFECTION

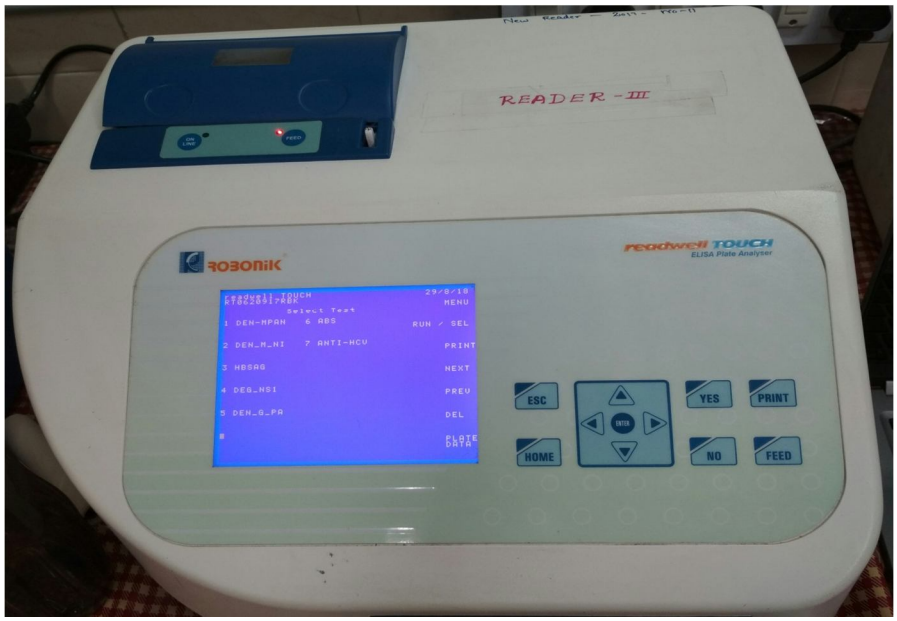




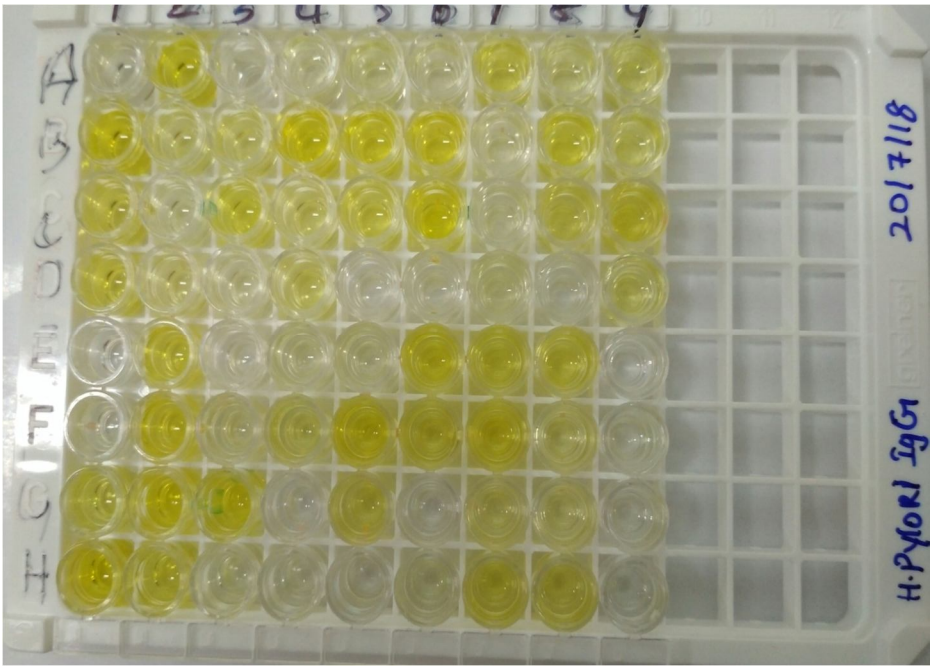
4. RAPID UREASE TEST - CONTROL

RAPID UREASE TEST - POSITIVE

5. ELISA READER & WASHER



6. H. pylori IgG ELISA



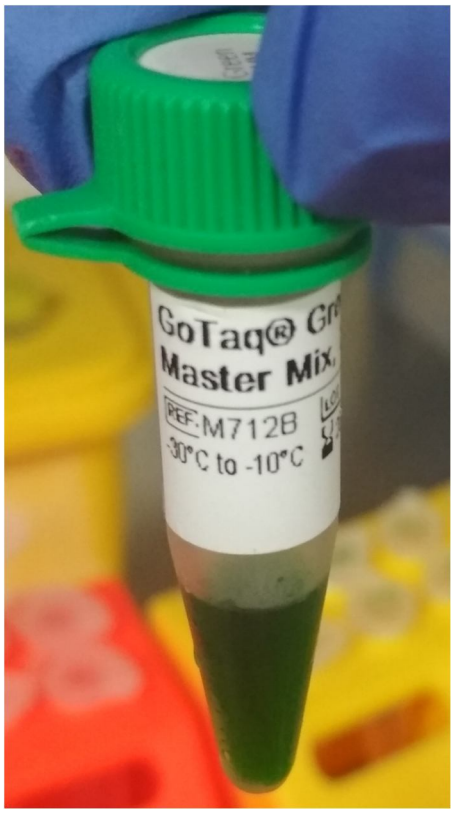
7. TISSUE GRINDING – MORTOR AND PESTLE

8. CENTRIFUGE

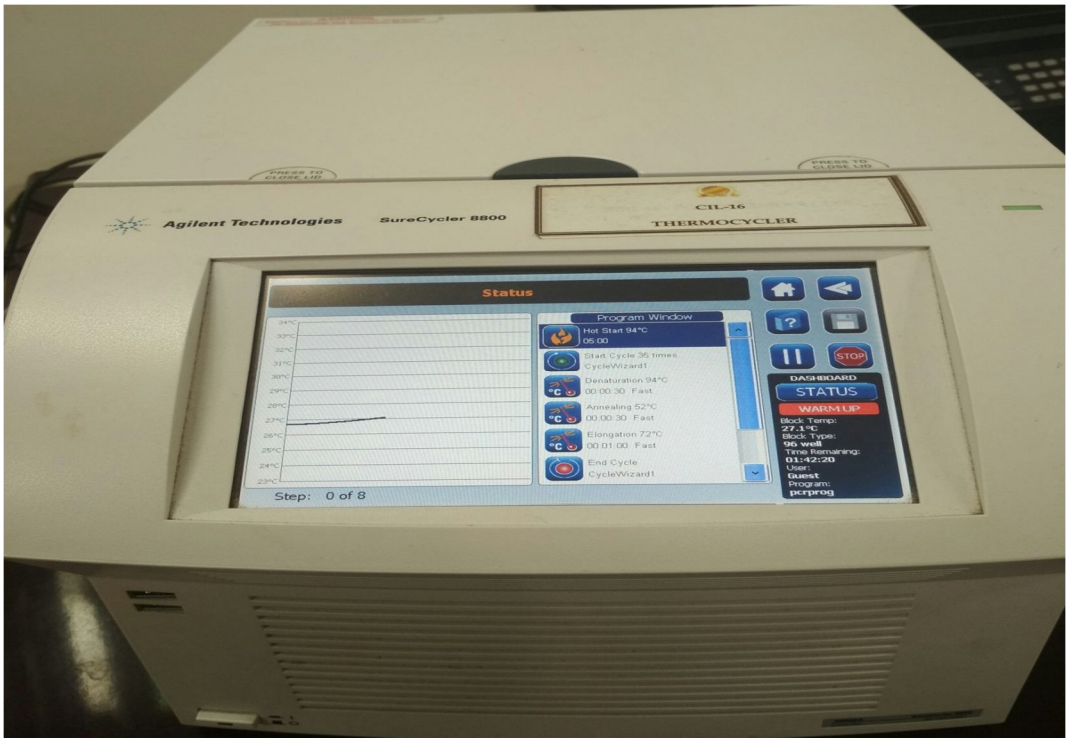
THERMOMIXER - VORTEX

9. AUTOMATIC DNA EXTRACTION MACHINE

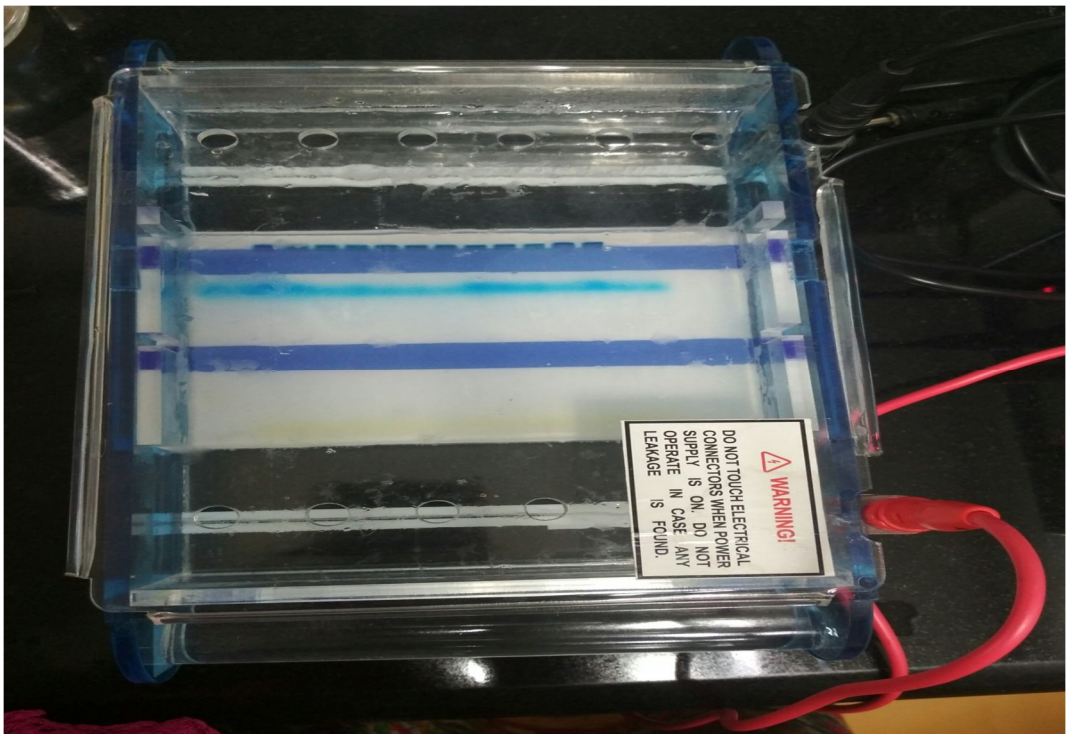
Taq Polymerase – Master Mix



10. PCR MACHINE

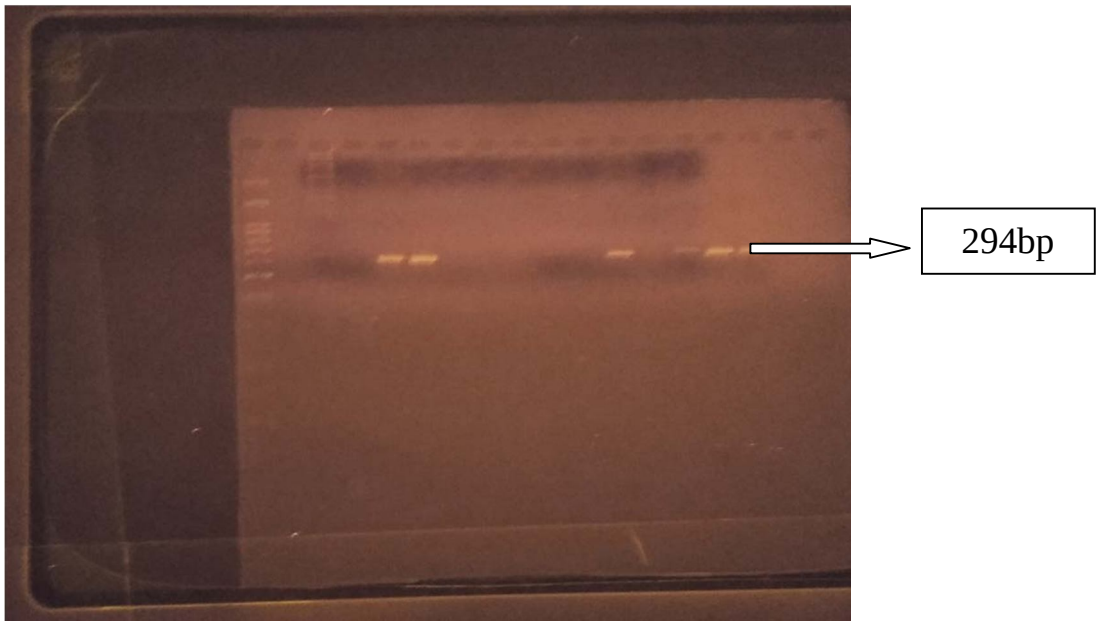


GEL ELECTROPHORESIS



11. UV SPECTROPHOTOMETER

GEL ELECTROPHORESIS



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Annexures

APPENDIX - I

LIST OF ABBREVIATIONS

H.pylori	-	Helicobacter pylori
PUD	-	Peptic ulcer disease
Ca stomach	-	Carcinoma stoma
Cag A gene	-	Cytotoxin associate
Vac gene	-	Vacuolating gene
PAI	-	Pathogenicity island
PPIs	-	Proton pump inhibitors
RUT	-	Rapid urease test
HPE	-	Histopathological examination
ELISA	-	Enzyme linked immunosorbant assay
HpSA	-	Helicobacter pylori stool antigen test
LPS	-	Lipopolysaccharides
Fla A	-	Flagellin A
b. i. d	-	Two times / day
Q. i. d	-	Four times / day
Ure A	-	Urease A gene
CLSI	-	Clinical and Laboratory Standard Institute
Hsp	-	Heat shock proteins
Hcp	-	Helicobacter cysteine rich protein
RT-PCR	-	Reverse transcriptase Polymerase chain reaction

CLO Test	-	Campylobacter like organism test
UBT	-	Urea Breath Test
IL _ 1	-	Interleukins – 1
IgG	-	Immunoglovulin G
NSAIDs	-	Non steroidal anti inflammatory drugs
Sab A	-	Sialic acid binding protein
Bab A	-	blood group antigen binding ahesin
MALT	-	Mucosa associated lymphoid tissue
GC content	-	Guanine Cytosine content
E test	-	Epsilometer test

APPENDIX II

A).STAINS AND REAGENTS

Gram staining:

- Methyl violet(2%)-10g of Methyl violet in 100 ml Absolute alcohol in 1 litre of Distilled water.(primary stain)
- Grams Iodine-10g Iodine in 20 g KI (fixative)
- Acetone-Decolourizing agent.
- Carbolfuchsin(1%)-Secondary stain.

Giemsa staining:

- 80% Methanol – Fixative
- Himedia Giemsa stain (undiluted) – Methylene blue aqueous solution, azure B and Preservative / (Acetone / dimethyl formamide / water solvent) and buffer.

B.MEDIA USED

1. Skirrow's Campylobacter Medium

I.	Campylobacter agar base	:	39.5 gm
II.	Distilled water	:	1 litre
III.	Skirrow・s supplement	:	1 vial consisting of
		:	10 mg Vancomycin

2. Chocolate Agar

Peptone	:	1.0 gm
Meat Extract	:	1.0 gm
Sodium chloride	:	0.5 gm
Agar agar	:	2.0 gm
Distilled water	:	100 ml
Defibrinated sheep blood	:	10%
Polymyxin B	:	2500 IU
Trimethoprim	:	50 ml

Sheep blood was used as horse blood was not available.

Amphotericin B 5mg /L and 10% defibrinated blood was used .

3. Phosphate Buffered Saline (PBS)

Ingredients	Grams/ litre
Sodium chloride	8.0
Potassium chloride	0.2
Disodium hydrogen phosphate	1.15
Potassium dihydrogen phosphate	0.2
Distilled water	1 litre

pH was adjusted to 7.4

The above ingredients were dissolved in sterile water and then filtered using filter paper.

ANNEXURE – I

INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE, CHENNAI 600 003

EC Reg.No.ECR/270/Inst./TN/2013
Telephone No.044 25305301
Fax: 011 25363970

CERTIFICATE OF APPROVAL

To
Dr.S.Viji
I Year PG in MD Microbiology
Institute of Microbiology
Madras Medical College
Chennai 600 003

Dear Dr.S.Viji,

The Institutional Ethics Committee has considered your request and approved your study titled **"A STUDY ON DIAGNOSIS OF HELICOBACTER PYLORI INFECTION BY CULTURE AND MOLECULAR METHODS FROM GASTRIC BIOPSY SPECIMENS AND SEROLOGICAL ASSAYS IN PATIENTS WITH PEPTIC ULCER DISEASE "** - **NO.08032017(I)**

The following members of Ethics Committee were present in the meeting hold on **02.03.2017** conducted at Madras Medical College, Chennai 3

1.Dr.C.Rajendran, MD.,	:Chairperson
2.Dr. K.Narayanasamy,MD,DM.,Dean(FAC), MMC,Ch-3	:Deputy Chairperson
3.Prof.Sudha Seshayyan,MD., Vice Principal,MMC,Ch-3	: Member Secretary
4.Prof.S.Suresh, MS, Prof. of Surgery,MMC,Ch-3	: Member
5.Prof.Baby Vasumathi,MD.,Director, Inst. of O & G	: Member
6.Prof.K.Ramadevi,MD.,Director,Inst.of Bio-Che,MMC,Ch-3	: Member
7.Prof.R.Padmavathy, MD, Director,Inst.of Pathology,MMC,Ch-3	: Member
8.Tmt.J.Rajalakshmi, JAO,MMC, Ch-3	: Lay Person
9.Thiru S.Govindasamy, BA.,BL,High Court,Chennai	: Lawyer
10.Tmt.Arnold Saulina, MA.,MSW.,	:Social Scientist

We approve the proposal to be conducted in its presented form.

The Institutional Ethics Committee expects to be informed about the progress of the study and SAE occurring in the course of the study, any changes in the protocol and patients information/informed consent and asks to be provided a copy of the final report.

Member Secretary – Ethics Committee

MEMBER SECRETARY
INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE

ANNEXURE – II

PROFORMA

Name: IP NO:

Age/sex Ward

Occupation; Income;

Address ; phone no;

- PRESENTING COMPLAINTS:

Epigastricpain: Vomiting\Nausea: Dyspepsia:

Haematemesis : Malena : Loss of weight:

Loss of appetite: others:

- PERSONAL HISTORY:

Alcohol: Smoking: H\o drug intake:

Diet habits: previous H\o gastric surgery:

- GENERAL EXAMINATION:

Consciousness: Built: Nourishment:

Pallor: Jaundice: Cyanosis:

Clubbing: Pedaledema: Lymphadenopathy:

Pulse: Bp: Respiratoy Rate:

Temperature:

- SYSTEMIC EXAMINATION:

Abd : Other systems:

- INVESTIGATIONS:

UGI Scopy : USG Abd: HPE;

RUT; Gram stain; Serology; PCR;

ANNEXURE – III

CONSENT FORM

STUDY TITLE : A study on diagnosis of Helicobacter pylori infection by culture and molecular methods from gastric biopsy specimens and serological assays in patients with peptic ulcer disease.

I ……, hereby give consent to participate in the study conducted by Dr.S. VIJI, Post graduate at Institute of Microbiology, Madras Medical College, Chennai and to use my personal clinical data and the result of investigations for the purpose of analysis and to study the nature of the disease, I also give consent to give my clinical Specimen (Gastric biopsy tissue and blood) for further investigations. I also learn that there is no additional risk in this study. I also give my consent for my investigator to publish the data in any forum or journal.

Signature/ Thumb impression;

Of the patient/ relative

Place
Date

Patient Name & Address:

Signature of the investigator:

Signature of guide:

ANNEXURE – IV

INFORMATION SHEET

STUDY TITLE : A study on diagnosis of Helicobacter pylori infection by culture and molecular methods from gastric biopsy specimens and serological assays in patients with peptic ulcer disease.

INVESTIGATOR : **Dr. S. VIJI,**
Post Graduate,
Institute of Microbiology,
Madras Medical College,
Chennai – 600003.

GUIDE : **Dr.Umadevi . U. M.D.,**
Professor,
Institute of Microbiology,
Madras Medical College,
Chennai – 600003.

In recent times, H.pylori infection is more common in developing countries among Low socio economic status. Since its an microaerophilic negative bacilli, only limited identification methods are available. So I am going to detect the prevalence of H.pylori infection among the clinical diagnosis of gastroduodenal disease patients subjected to endoscopy procedure in the tertiary hospital. I am going to collect clinical specimens such as gastric biopsy tissue and blood and process them accordingly. 120 patients are included in this study after getting informed consent. This study is entirely voluntary and patient can withdraw any time from this study. Extra cost will not be incurred to the patients. Any doubt regarding this study will be willingly clarified. Results of the study will be published. In case of any doubt please contact Dr. S. Viji, Contact No:9976749172.

LEGENDS OF MASTER CHART

PUD	-	Peptic ulcer disease
Ca stomach	-	Carcinoma of stomach
U. P. G	-	Ulceroproliferate growth
Adenoca	-	Adenocarcinoma of stomach
Neg	-	Negative
NG	-	No Growth
P	-	Present / Positive
PH	-	Private Hospital
LOA	-	Loss of Appetite
LOW	-	Loss of Weight
Endo.No	-	Endoscopic number
RUT	-	Rapid urease test
HPE	-	Histopathological Examination
AST	-	Antibiotic Sensitivity Test
ELISA	-	Enzyme Linked Immunosorbent Assay
PCR	-	Polymerase chain Reaction
Dyspep	-	Dyspepsia
Haema	-	Haematemesis
Epigas	-	Epigastric pain

MASTER CHART

S. NO	AGE	SEX	dyspep	haema	epigast	LoA	Low	smoking	alcohol	diet	ENDO. NO	DIAG NOSIS	RUT	GRAM STAIN	GIEMSA STAIN	HPE.NO	DIAGNOSIS	CULTURE	AST	ELISA	PCR
1	63	M	P	P	P	P	P	P	P	mixed	4792	U.P.G	P		NEG	6295	ADENO CA STOMAC	NG		P	
2	60	M	P	P	P	P	P	P	P	mixed	4794	U.P.G			NEG	6296	PUD	NG		P	
3	54	M	P	P	P	P	P	P	P	mixed	4796	U.P.G		P	NEG	6297	CA STOMACH	NG			
4	65	M	P	P	P	P	P	P	P	mixed	4795	U.P.G			NEG	6966	PUD	NG			
5	73	M	P	P	P	P	P	P	P	mixed	5038	PUD			NEG	6301	CA STOMACH	NG		P	
6	55	M	P	P	P	P	P	P	P	mixed	5040	U.P.G			NEG	6303	CA STOMACH	NG			
7	25	M	P	P	P	P	P	P	P	mixed	5182	PUD			NEG		PUD	NG			
8	17	M	P	P	P	P	P	P	P	mixed	5186	U.P.G		P	NEG	7528	CA STOMACH	NG			
9	72	F		P	P					mixed	5189	PUD			NEG		PUD	NG			
10	58	M	P	P	P	P	P	P	P	mixed	5192	PUD			NEG		PUD	NG			
11	13	F			P					mixed	6210	PUD			NEG		PUD	NG			
12	60	M			P				P	mixed	6212	PUD			NEG	7548	PUD	NG			
13	53	M	P	P	P	P	P	P	P	mixed	6220	U.P.G			NEG	7542	ADENO CA STOMACH	NG		P	
14	50	M							P	mixed	2569	U.P.G	P		NEG	7485	ADENO CA STOMACH	NG		P	
15	35	F								mixed	2568	PUD	P		NEG	7486	PUD	NG			p
16	24	M	P		P	P	P	P	P	mixed	2570	PUD		P	NEG	7488	PUD	NG			
17	59	M	P		P	P	P	P	P	mixed	399	PUD	P	P	NEG		PUD	NG			
18	71	F			P					mixed	381	PUD	P	P	NEG		PUD	NG			
19	60	F			P					mixed	395	ANTRAL GROWTH	P	P	NEG	7520	CA STOMACH	NG			
20	41	M			P				P	mixed	2179	PUD	P		NEG		PUD	NG			
21	49	M	P		P	P	P	P	P	mixed	4659	U.P.G			NEG	7526	CA STOMACH	NG		P	p
22	48	F								mixed	2651	U.P.G	P		NEG	7531	CA STOMACH	NG		P	
23	47	M	P	P	P	P	P		P	mixed	2636	PUD			NEG		PUD	NG			
24	63	F	P		P	P	P			mixed	2645	PUD	P	P	NEG	7536	PUD	NG		P	p
25	63	M	P		P	P	P		P	mixed	2229	PUD	P		NEG		PUD	NG			
26	43	F	P		P	P	P			veg	2226	PUD	P		NEG		PUD	NG			
27	48	F	P	P	P	P	P			mixed	2664	PUD	P		NEG		PUD	NG		P	
28	28	M	P	P	P	P	P	P		mixed	2656	PUD	P		NEG		CA STOMACH	NG			

S. NO	AGE	SEX	dyspep	haema	epigast	LoA	Low	smoking	alcohol	diet	ENDO. NO	DIAG NOSIS	RUT	GRAM STAIN	GIEMSA STAIN	HPE.NO	DIAGNOSIS	CULTURE	AST	ELISA	PCR
29	60	M	P	P	P	P	P	P		mixed	2666	PUD	P	P	NEG	P.HOS	ADENO CA STOMACH	NG		P	
30	51	M	P		P	P	P	P	P	mixed	2676	U.P.G	P		NEG	7836	ADENO CA STOMACH	NG			
31	65	M						P	P	mixed	2833	U.P.G	P		NEG	7912	PUD	NG		P	p
32	60	M	P	P	P	P	P	P	P	veg	6387	U.P.G	P	P	NEG	8510	PUD	NG			
33	41	F								mixed	5635	PUD	P		NEG	8539	ADENO CA STOMACH	NG		P	p
34	62	M						P	P	mixed	2956	U.P.G			NEG	8871	CA STOMACH	NG			
35	59	M	P		P	P	P	P	P	mixed	2949	U.P.G			NEG	8876	PUD	NG		P	
36	32	M						P	P	mixed	2607	PUD		P	NEG	8999	CA STOMACH	NG			
37	70	M						P	P	mixed	7027	U.P.G			NEG	9002	PUD	NG			p
38	65	M	P	P	P	P	P	P	P	mixed	370	PUD			NEG		PUD	NG			
39	40	F	P		P	P	P			mixed	7028	PUD			NEG		PUD	NG			
40	24	M						P	P	mixed	2570	PUD			NEG		PUD	NG			
41	58	M								veg	7241	PUD			NEG		CA STOMACH	NG			
42	75	F								veg	2724	U.P.G			NEG	6427	CA STOMACH	NG			
43	32	M	P	P	P	P	P			mixed	2726	U.P.G			NEG	5964	CA STOMACH	NG		p	
44	60	F								mixed	2759	U.P.G			NEG	9581	PUD	NG			
45	56	M	P		P	P	P	P	P	mixed	3268	PUD	P	P	NEG		CA STOMACH	NG			
46	49	F	P		P	P	P			mixed	2785	U.P.G	P	P	NEG	6485	CA STOMACH	NG			p
47	60	F	P		P	P	P			mixed	3163	U.P.G			NEG	9610	CA STOMACH	NG			
48	70	M	P		P	P	P			mixed	3165	U.P.G			NEG	7382		NG			
49	52	M	P	P	P	P	P	P	P	mixed	3168	U.P.G			NEG	4665	CA STOMACH	NG			
50	80	F	P		P	P	P			mixed	3206	U.P.G		P	NEG	9877	CA STOMACH	NG			
51	34	M	P		P	P	P			mixed	3212	PUD			NEG		PUD	NG			
52	56	M	P		P	P	P	P	P	mixed	3310	U.P.G	P	P	NEG	9925	CA STOMACH	NG			
53	52	M	P							mixed	P.H	U.P.G	P		NEG	PH	CA STOMACH	NG			
54	68	M	P		P	P	P	P	P	mixed	19	U.P.G	P		NEG	1225	CA STOMACH	NG			
55	75	M	P		P	P		P	P	mixed	25	U.P.G	P		NEG	1230	ADENO CA STOMACH	NG			
56	63	M	P	P	P	P		P	P	mixed	106	U.P.G	P	P	NEG	1312	ADENO CA STOMACH	NG			
57	57	M	P		P	P	P	P	P	mixed	PH	U.P.G	P		NEG	PH	ADENO CA STOMACH	NG			

S. NO	AGE	SEX	dyspep	haema	epigast	LoA	Low	smoking	alcohol	diet	ENDO. NO	DIAG NOSIS	RUT	GRAM STAIN	GIEMSA STAIN	HPE.NO	DIAGNOSIS	CULTURE	AST	ELISA	PCR
58	52	M	P	P	P	P	P	P	P	mixed	470	U.P.G	P		NEG	1495	ADENO CA STOMACH	NG			
59	55	F	P	P	P	P				mixed	69	U.P.G	P		NEG	726	CA STOMACH	NG			
60	55	M	P		P	P	P	P	P	mixed	460	U.P.G	P	P	NEG	1431	ADENO CA STOMACH	NG			
61	64	M	P		P	P	P	P	P	mixed	483	U.P.G	P		NEG	1286	CA STOMACH	NG			p
62	68	M	P		P	P	P	P	P	mixed	PH	U.P.G	P		NEG	PH	ADENO CA STOMACH	NG			
63	63	M	P		P	P	P	P	P	mixed	PH	U.P.G	P	P	NEG	PH	ADENO CA STOMACH	NG			
64	59	M	P		P	P	P	P	P	mixed	3611	U.P.G	P		NEG	1222	ADENO CA STOMACH	NG		P	p
65	40	M	P	P	P	P	P			mixed	PH	U.P.G	P		NEG	PH	ADENO CA STOMACH	NG		P	p
66	57	M	P	P	P	P	P			mixed	1269	U.P.G	P	P	NEG	1735	CA STOMACH	NG			
67	52	F	P		P	P	P			mixed	100	U.P.G	P		NEG	2028	CA STOMACH	NG		P	
68	50	F	P		P	P	P			mixed	PH, 1270	PUD	P	P	NEG	2029	CA STOMACH	NG		P	
69	37	M	P	P	P	P	P	P	P	mixed	622	U.P.G	P	P	NEG	1569	ADENO CA STOMACH	NG		P	p
70	68	M	P	P	P	P	P	P	P	mixed	9636	PUD	P		NEG	7883	PUD	NG			
71	27	M	P		P	P	P	P	P	mixed	600	PUD			NEG	1636	PUD	NG			
72	30	M	P	P	P	P	P	P	P	mixed	602	U.P.G	P		NEG	1638	CA STOMACH	NG		P	p
73	49	M	P		P	P	P	P	P	mixed	524	U.P.G	P		NEG	1309	CA STOMACH	NG		P	p
74	73	M	P		P	P	P	P	P	mixed	689	U.P.G	P	P	NEG	1546	ADENO CA STOMACH	NG		P	p
75	32	M	P		P	P	P	P	P	mixed	692	U.P.G			NEG	1549	CA STOMACH	NG			
76	55	M	P	P	P	P	P	P	P	mixed	695	U.P.G	P		NEG	2236	ADENO CA STOMACH	NG			p
77	48	M	P		P	P	P	P	P	mixed	650	U.P.G		P	NEG	2238	ADENO CA STOMACH	NG			
78	62	M	P		P	P	P	P	P	mixed	773	U.P.G	P		NEG	2558	ADENO CA STOMACH	NG		P	
79	39	M	P		P	P	P	P	P	mixed	1961	U.P.G	P		NEG	2560	ADENO CA STOMACH	NG			p
80	52	M	P		P	P	P	P	P	mixed	3168	U.P.G	P	P	NEG	2562	ADENO CA STOMACH	NG			p
81	52	M	P		P	P	P	P	P	mixed	121	U.P.G			NEG	2575	ADENO CA STOMACH	NG		P	p
82	60	M	P	P	P	P	P	P	P	mixed	134	U.P.G	P		NEG	394	ADENO CA STOMACH	NG			

S. NO	AGE	SEX	dyspep	haema	epigast	LoA	Low	smoking	alcohol	diet	ENDO. NO	DIAG NOSIS	RUT	GRAM STAIN	GIEMSA STAIN	HPE.NO	DIAGNOSIS	CULTURE	AST	ELISA	PCR
83	65	M	P		P	P	P	P	P	mixed	728	U.P.G	P	P	NEG	2578	ADENO CA STOMACH	NG			p
84	70	M	P	P	P	P	P	P	P	mixed	155	U.P.G	P		NEG	2841	ADENO CA STOMACH	NG		P	p
85	46	M	P		P	P	P	P	P	mixed	573	U.P.G		P	NEG	2174	CA STOMACH	NG			p
86	56	M	P		P	P	P	P	P	mixed	874	U.P.G	P	P	NEG	2435	CA STOMACH	NG		P	
87	67	M	P		P	P	P	P	P	mixed	PH,876	U.P.G			NEG	PH	ADENO CA STOMACH	NG			
88	40	F	P		P	P	P			mixed	PH	U.P.G			NEG	PH	CA STOMACH	NG			
89	57	M	P		P					mixed	6100	U.P.G	P		NEG	2936	ADENO CA STOMACH	NG			
90	50	M	P		P	P	P	P	P	mixed	PH	U.P.G	P		NEG	PH	ADENO CA STOMACH	NG			
91	40	M	P		P	P	P	P	P	mixed	880	U.P.G			NEG	3628	ADENO CA STOMACH	NG			
92	57	M	P		P	P	P	P	P	mixed	882	U.P.G	P	P	NEG	3391	CA STOMACH	NG			
93	65	M	P				P	P	P	VEG	180	U.P.G			NEG	3502	ADENO CA STOMACH	NG			
94	55	M	P			P	P	P	P	VEG	204	U.P.G	P		NEG	3506	CA STOMACH	NG			
95	42	M	P				P	P	P	VEG	206	U.P.G			NEG	3508	ADENO CA STOMACH	NG			
96	60	M	P			P	P	P	P	mixed	PH	U.P.G			NEG	PH	ADENO CA STOMACH	NG			
97	55	M	P		P		P	P	P	mixed	3410	U.P.G			NEG	PH	ADENO CA STOMACH	NG			
98	59	M	P	P	P		P			mixed	3508	U.P.G	P		NEG	3618	ADENO CA STOMACH	NG			
99	60	M	P		P	P	P	P	P	mixed	289	U.P.G	P		NEG	4890	CA STOMACH	NG			
100	57	M	P		P	P	P	P	P	mixed	416	U.P.G	P	P	NEG	4745	CA STOMACH	NG			
101	40	M	P	P	P	P	P	P	P	mixed	3906	U.P.G	P		NEG	4748	ADENO CA STOMACH	NG		P	
102	81	M	P		P	P	P	P	P	mixed	3588	U.P.G	P		NEG	5291	CA STOMACH	NG		P	
103	69	M	P		P	P	P	P	P	mixed	PH	U.P.G		P	NEG	PH	ADENO CA STOMACH	NG			
104	52	M	P	P	P	P	P	P	P	VEG	PH	U.P.G			NEG	PH	ADENO CA STOMACH	NG		P	
105	28	M	P		P	P	P	P	P	mixed	2655	PUD			NEG		PUD	NG			
106	28	F	P			P	P			mixed	1569	PUD			NEG		PUD	NG		P	
107	70	F	P			P	P			VEG	1545	PUD			NEG		PUD	NG			

S. NO	AGE	SEX	dyspep	haema	epigast	LoA	Low	smoking	alcohol	diet	ENDO. NO	DIAG NOSIS	RUT	GRAM STAIN	GIEMSA STAIN	HPE.NO	DIAGNOSIS	CULTURE	AST	ELISA	PCR
108	40	F	P			P	P			VEG	3856	PUD	P	P	NEG		PUD	NG			
109	64	F	P		P	P				mixed	3842	U.P.G	P	P	NEG	5293	ADENO CA STOMACH	NG			
110	32	F	P		P	P	P	P	P	mixed	1046	PUD			NEG		PUD	NG		P	
111	38	M	P		P	P	P	P	P	mixed	1585	PUD			NEG		PUD	NG			
112	50	M			P	P	P	P	P	mixed	1582	PUD			NEG		PUD	NG			
113	50	M	P		P	P	P	P	P	mixed	1592	PUD	P	P	NEG		PUD	NG		P	p
114	70	M			P	P	P	P	P	mixed	1384	U.P.G	P	P	NEG	5296	ADENO CA STOMACH	NG			
115	30	F	P	P	P	P	P			mixed	1589	PUD			NEG		PUD	NG		P	p
116	46	M	P	P	P	P	P	P	P	mixed	1680	U.P.G			NEG	5796	ADENO CA STOMACH	NG			
117	60	M	P		P	P				mixed	696	U.P.G			NEG	5502	ADENO CA STOMACH	NG		P	
118	64	M			P	P	P	P	P	mixed	1692	U.P.G			NEG	4665	ADENO CA STOMACH	NG			
119	50	M	P	P	P	P	P	P	P	mixed	1702	U.P.G			NEG	5506	ADENO CA STOMACH	NG			
120	43	M	P			P	P			mixed	1699	U.P.G	P		NEG	5508	ADENO CA STOMACH	NG			